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CONTENTS

Comparative Evaluation of Epinephrine and Aminophylline as Bronchodilator Agents. Ross C. Kory, Robert A. Peiper, and Richard O. Sternlieb 729
The Induction of Respiratory Acidosis by Oxygen Breathing. Herman F. Froer, Charles I. Leftwich, and Hurley L. Motley 737
Histoplasmosis. A Review of One Hundred Consecutively Hospitalized Patients. Francis J. Curry and James A. Wise 749
The Fate of the Patient with Persistent Cavitation and Noninfectious Sputum ("Open-Negative") After Discharge from the Hospital. R. F. Corpe and F. A. Blalock 764
The Disc-Method Tuberculin Test. Sol Roy Rosenthal 778
Assay of the Fate of Mycobacteria in Cell and Tissue Cultures. John H. Hanes 789
The Effects of Extended Incubation on Propagability of Tubercle Bacilli. H. Z. Kennedy, H. M. Vandiviere, and H. S. Willis 802
The Effect of Chemotherapy on the Leukocytic Sensitivity to Tuberculin in Guinea Pigs Experimentally Infected with *Mycobacterium Tuberculosis* H37Rv. H. E. Hall and M. Scherago 815
Mycobacteria from Cold-Blooded Animals. Henry Vogel 823
Case Reports:
Silicosis of Ocher Workers. A Clinical and Anatomo-pathologic Study. A. D. Roche, D. Picard, and A. Verrières 839
Obstruction of the Superior Vena Cava Presumably Due to Histoplasmosis. Donald B. Miller, Sinclair T. Allen, Jr., and E. L. Amidon 848
Pyrazinamide-Induced Acute Yellow Atrophy of the Liver. Case Report and Post-Mortem Findings. Theodore N. Spengos and Rod Cubon 858
Notes:
Application of Hooke's Law to the Elastic Properties of the Lung. Robert Gilbert *et al.* .. 863
Short-Term Therapy in Experimental Tuberculosis. Florence K. FitzPatrick 867
Correspondence: "Sputum Conversion" and the Metabolism of Isoniazid. John Crofton 869
Books 872
American Trudeau Society:
Necrology 874
Notices 875
Abstracts 129

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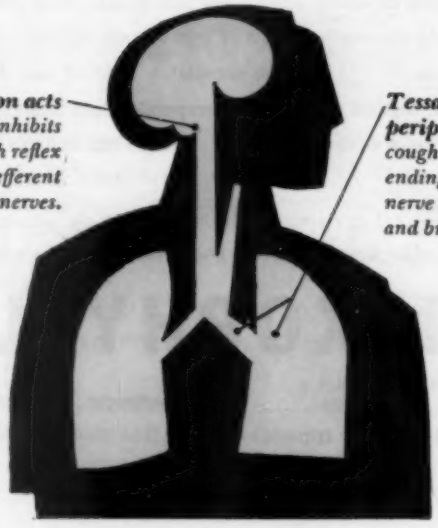
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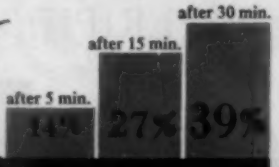

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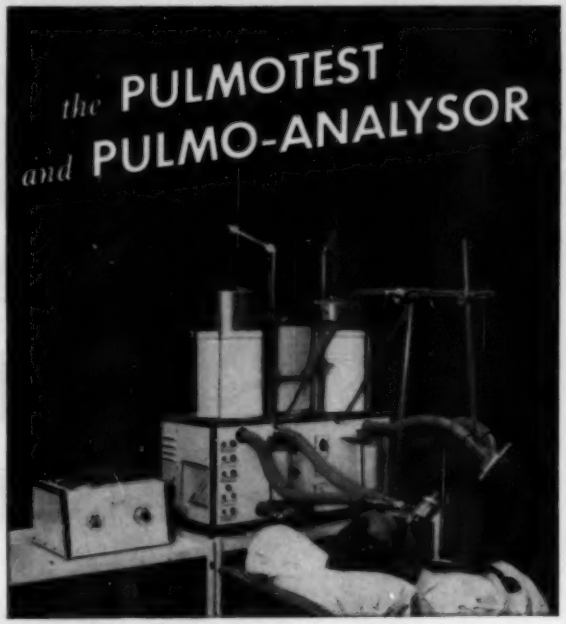

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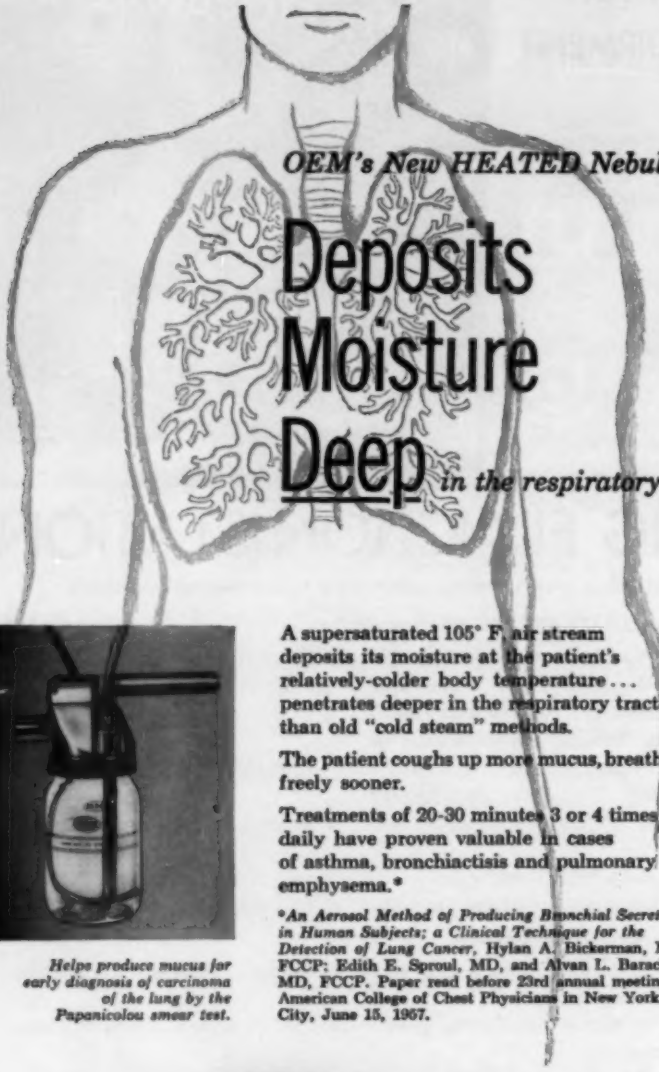

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1. Molthan, L., Cohen, R.V., and Zarafonetis, C.J.D.: Am. Rev. Tuber. *71*:220, 1955.
2. Cohen, R.V., Molthan, L., and Zarafonetis, C.J.D.: Dis. Chest *30*:418, 1956.

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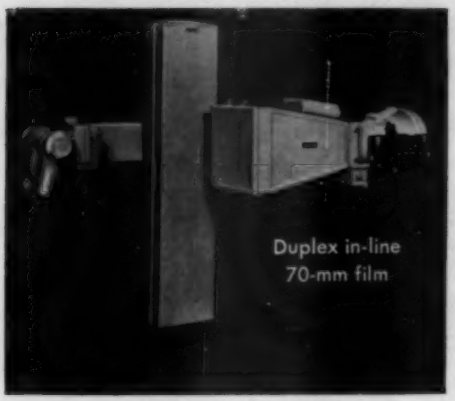

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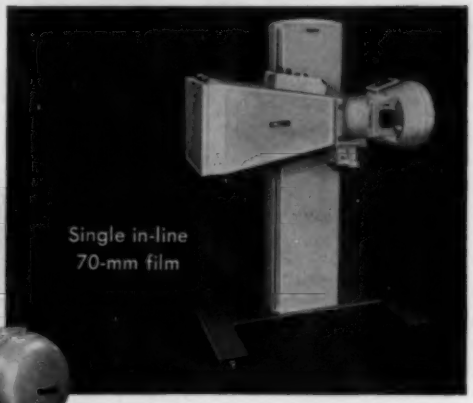

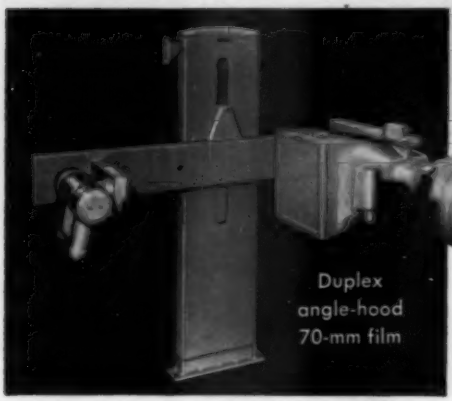

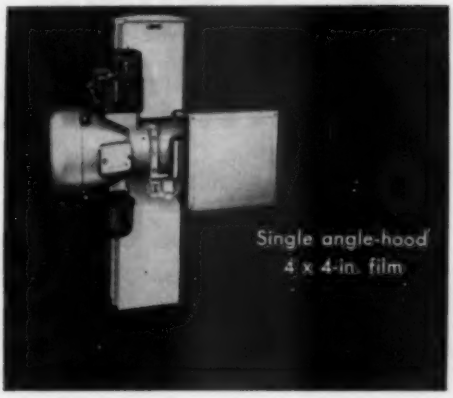

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 77 *May, 1958* NUMBER 5

COMPARATIVE EVALUATION OF EPINEPHRINE AND AMINOPHYLLINE AS BRONCHODILATOR AGENTS[1]

ROSS C. KORY, ROBERT A. PRIBEK, AND RICHARD O. STERNLIEB

With the technical assistance of Helen C. Delfeld and Jaqueline Owenby

(Received for publication December 2, 1957)

INTRODUCTION

Bronchodilator agents play an important role in the present-day diagnosis and management of the bronchospasm which is seen in bronchial asthma, pulmonary emphysema, and chronic bronchitis. Epinephrine and aminophylline are commonly used and widely accepted as bronchodilator agents. Although the bronchodilator actions of subcutaneous epinephrine and intravenous aminophylline have been evaluated individually by both clinical and laboratory studies, a review of the literature has failed to reveal any report in which the two drugs were compared by objective measurements in the same patient. It is the purpose of this report to show by spirometric measurements the relative bronchodilator effect of subcutaneous epinephrine and intravenous aminophylline in the same patient under standard conditions.

Epinephrine was first reported as a useful bronchodilator agent by Solis-Cohen in 1900 (1). During the ensuing quarter of a century the drug was employed sporadically as a clinical bronchodilator. In 1925, Mudd (2) demonstrated an improvement in vital capacity following subcutaneous epinephrine in three patients with acute bronchial asthma. Several subsequent evaluations (3–7) have substantiated the potent bronchodilator action of epinephrine.

Aminophylline was first mentioned in the therapy of bronchial asthma by Efron in 1936 (8). Its clinical value in the relief of bronchospasm was pointed out in 1937 by Herrmann and Aynesworth (9), who reported prompt and persistent relief in thirty-one of forty-one cases of status asthmaticus. Subsequent to their reports a number of clinical and pharmacologic studies confirmed the efficacy of aminophylline as a bronchodilator (10–19). Several more recent investigations have compared the bronchodilator effect of aminophylline with that of newer preparations (20–23).

[1] From the Cardiopulmonary Laboratory, Medical and Research Services, Wood Veterans Administration Hospital; and the Department of Medicine, Marquette University School of Medicine, Milwaukee, Wisconsin.

Material and Methods

Thirty-eight male patients were selected for study on the basis of clinically suspected bronchospasm. These included patients with bronchial asthma, pulmonary emphysema, and chronic bronchitis. None of the patients at the time of study were receiving epinephrine, aminophylline, corticotropin, or adrenal cortical steroids.

Vital capacity and maximal breathing capacity tests were performed initially on each patient. After these base-line studies were obtained, the patient was given either 0.3 mg. of epinephrine subcutaneously or 0.5 gm. of aminophylline in 20 ml. of normal saline intravenously. Repeat measurements of vital capacity and maximal breathing capacity were done ten and thirty minutes after injection in all cases. When continued improvement occurred during the first thirty minutes, studies were also done sixty minutes after drug administration. Within one week the patient was re-tested with the other drug at the same time of day under similar conditions.

The following spirometric measurements were employed in this study:

Total vital capacity (VC) = total volume of air expired after a maximal inspiration, in liters.

0.5-second VC = volume of air expired in the first 0.5 second of the vital capacity effort, in liters.

One-second VC = volume of air expired in the first second of the vital capacity effort, in liters.

Three-second VC = volume of air expired during the first 3 seconds of the vital capacity effort, in liters.

The maximal expiratory flow rate (MEFR) (24) = the velocity of air flow in liters per second between the first 200 and 1,200 cc. of air expired during the vital capacity test.

Maximal midexpiratory flow rate (MMEF) (25) = velocity of air flow in liters per second during the middle half of the volume expired during the vital capacity test.

The maximal breathing capacity (MBC) = the volume of air expressed in liters per minute, which can be moved during a 12-second period of maximal voluntary hyperventilation.

All gas volumes are corrected to 37° C., ambient pressure, saturated (BTPS).

The vital capacity was measured on a Collins 13.5 liter respirometer with kymograph speeds of either 160 mm. or 480 mm. per minute. The best of three vital capacity efforts was used for the base-line test, but after the drug administration the better of two determinations was accepted unless a wide discrepancy between these two efforts was noted. The maximal breathing capacity was also performed on a 13.5 liter Collins respirometer with a 12-second period of voluntary hyperventilation in each case. Again the best of three trials was used as a base line, but the better of two was accepted after drug administration unless a wide variation was seen, in which case additional trials were run.

It was recognized from the onset of the study that a valid comparison of the two drugs could be obtained only if the patient's pulmonary status was essentially similar at the time of evaluation of each drug. In order to achieve this similarity, the patients were selected on the basis of their ability and willingness to undergo repeated spirometric testing. Each patient was tested at the same time of day, by the same person, with the same instrument, in the same air-conditioned room.

Results

During the course of the study, it was frequently observed that an individual patient on one day would exhibit initial spirometric values which varied con-

siderably from the initial values obtained at the time of testing the other drug. Because of these variations it was considered justifiable to include only those measurements in which the two initial base-line values were within 10 per cent of each other.

A total of 38 patients were tested, but 12 of these were excluded entirely because of variations in the base-line values of greater than 10 per cent. Such variations also made it necessary to eliminate certain specific measurements from those 26 patients included in the study.

For each determination, the maximal increase obtained after each drug is expressed as the percentage increase over the value obtained before the administration of the bronchodilator. The mean maximal percentage increases for the

TABLE 1

MEAN MAXIMAL PERCENTAGE INCREASES IN 7 VENTILATORY PARAMETERS AFTER EPINEPHRINE AND AFTER AMINOPHYLLINE

Determination	Number of Valid Comparative Tests	Mean Maximal Increase in Per Cent		Standard Deviation for Differences Between the Two Drugs
		After 0.3 Mg. of Epinephrine	After 0.5 Gm. of Aminophylline	
Total vital capacity	23	16	10	2.4*
0.5 second vital capacity	19	19	11	5.6
1 second vital capacity	23	17	13	3.5
3 second vital capacity	23	15	12	2.1
Maximal expiratory flow rate (MEFR)	11	34	26	6.2
Maximal mid-expiratory flow rate (MMEF)	11	15	9	6.5
Maximal breathing capacity	20	20	10	2.4†

Percentage values were transformed into angles (angle = arc − sin $\sqrt{\text{percentage}}$) for mean values and t tests.

* $p < 0.05$

† $p < 0.03$

TABLE 2

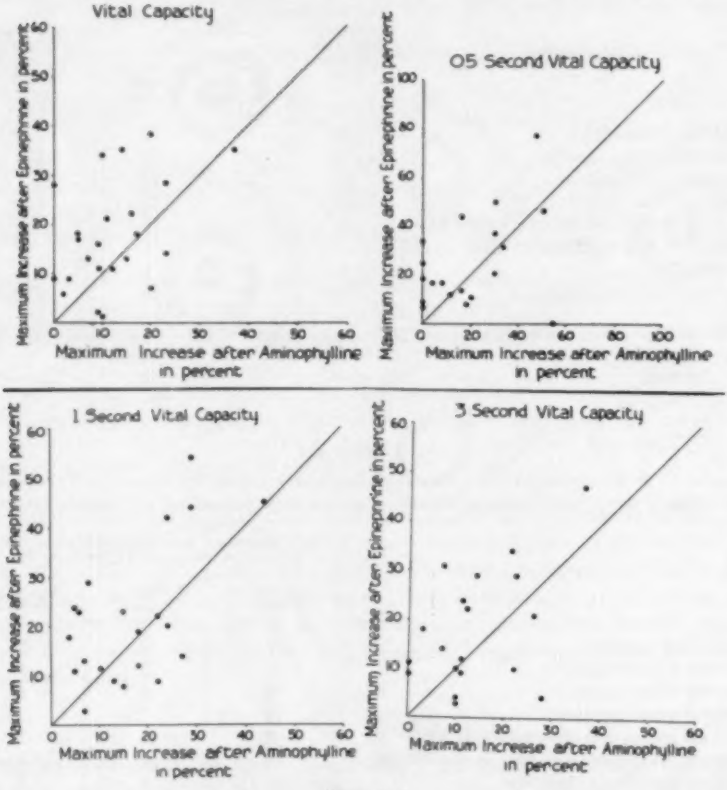
NUMBER OF PATIENTS SHOWING A GREATER BRONCHODILATOR RESPONSE TO EPINEPHRINE COMPARED WITH THE NUMBER SHOWING A GREATER RESPONSE TO AMINOPHYLLINE

Determination	Greater Response to Epinephrine	Greater Response to Aminophylline	Equal Response to the Two Drugs
	Number of Patients	Number of Patients	Number of Patients
Total vital capacity	16	7	0
0.5 second vital capacity	11	7	1
1 second vital capacity	15	8	1
3 second vital capacity	13	7	3
Maximal expiratory flow rate (MEFR)	6	4	1
Maximal mid-expiratory flow rate (MMEF)	6	4	1
Maximal breathing capacity	13	6	1

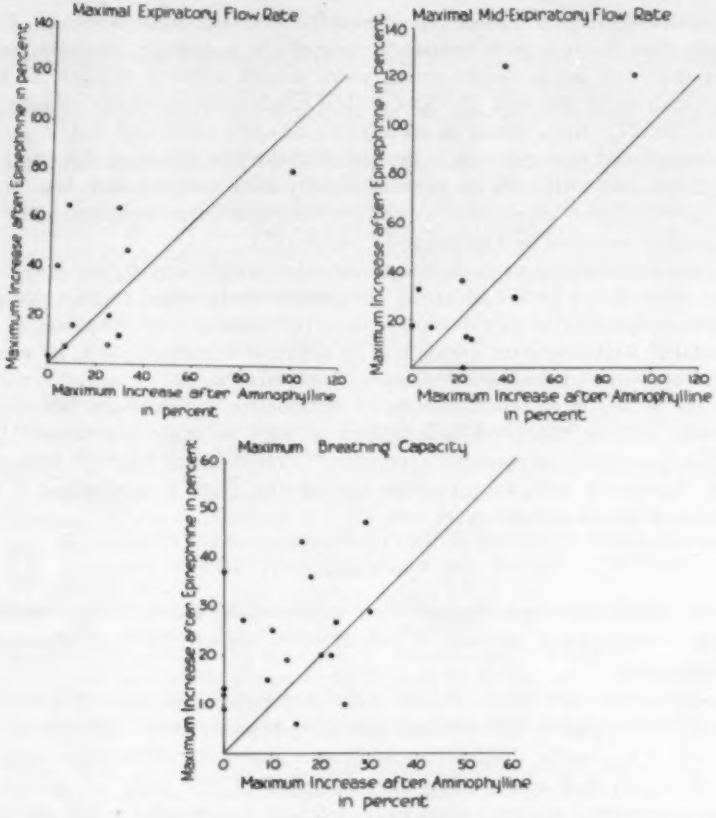
various measurements are outlined in table 1. It may be seen that in each of the seven determinations, 0.3 mg. of subcutaneous epinephrine produced a greater mean increase than did 0.5 gm. of intravenous aminophylline. These were statistically significant for the total vital capacity measurements ($P < 0.05$) and the maximal breathing capacity ($P < 0.03$).

The number of patients showing a greater response to epinephrine and the number showing a greater response to aminophylline are presented in table 2. For each measurement, epinephrine resulted in a greater response in more patients than did aminophylline. In figures 1 through 7, the maximal percentage increases after epinephrine are compared graphically with the maximal percentage increases after aminophylline. These graphs further illustrate that epinephrine was not only more effective in a greater number of patients, but that the degree of response in individual patients was greater than with aminophylline.

The duration of bronchodilator activity with the two drugs was compared


Vital Capacity
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent
0.5 Second Vital Capacity
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent
1 Second Vital Capacity
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent
3 Second Vital Capacity
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent

FIGS. 1–4.


Maximal Expiratory Flow Rate
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent
Maximal Mid-Expiratory Flow Rate
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent
Maximum Breathing Capacity
Maximum Increase after Epinephrine in percent
Maximum Increase after Aminophylline in percent

FIGS. 5–7.

FIGS. 1–7. These graphs are prepared in similar fashion with the abscissae representing the maximal per cent increase following an intravenous dose of 0.5 gm. of aminophylline and the ordinates representing the maximal per cent increase following 0.3 mg. of subcutaneous epinephrine. The diagonal lines have been arbitrarily inscribed to represent the equal response of the patient to each drug. Those points falling *above* the line indicate a greater response to epinephrine and those *below* the line a greater response to aminophylline.

over a sixty-minute period in most patients. Studies beyond sixty minutes were attempted in the first few patients, but were abandoned because of patient fatigue.

DISCUSSION

As the subcutaneous administration of epinephrine is more convenient and is safer than the intravenous administration of aminophylline, epinephrine would need only to equal aminophylline in bronchodilator activity to be more useful as a bronchodilator drug. Studies of the bronchodilator activity of epinephrine

have with few exceptions employed doses of 0.5 to 1.0 mg. subcutaneously. These dosages often result in such undesirable side effects as anxiety, dizziness, pallor, palpitation, and headache. In recent years smaller doses of epinephrine have been found to be effective clinically with a much lower incidence of such side effects (26, 27). Intravenous aminophylline has the additional side effects of hypotension and syncope. It is of interest that the side effects in this study, although minimal with both drugs, were slightly more frequent with aminophylline. An even higher incidence of side effects following intravenous aminophylline was recently reported by Cander and Comroe (21).

Recent investigators have usually employed aminophylline as the "standard" in the assay of new bronchodilators. The present study would suggest that subcutaneous epinephrine might well replace intravenous aminophylline as the "standard" bronchodilator because of its superior bronchodilation, its ease of administration, and the lesser incidence of side effects.

In the detection of bronchospasm by spirometric testing, most laboratories currently employ nebulized bronchodilators such as isopropylarterenol (Isuprel®) and racemic epinephrine (Vaponefrin®). These agents have not been compared objectively with subcutaneous epinephrine. Such a comparison is now being made in this laboratory.

Summary

Serial spirometric measurements were employed for the objective evaluation of the bronchodilator activity of subcutaneous epinephrine and intravenous aminophylline.

Subcutaneous epinephrine showed significant superiority over aminophylline in total vital capacity and maximal breathing capacity tests. Epinephrine was equal to, if not better than, aminophylline in improving timed vital capacities and expiratory flow rates. Under the conditions of this study no appreciable difference between the two drugs in duration of bronchodilator activity could be detected. The side effects encountered in this study were minimal but slightly more frequent with intravenous aminophylline.

Sumario

Valuación Comparativa de la Epinefrina y la Aminofilina como Broncodilatadores

Empleáronse mediciones espirométricas en serie para la valuación objetiva de la actividad broncodilatadora de la epinefrina por vía subcutánea y la aminofilina por vía endovenosa.

La epinefrina subcutánea reveló notable superioridad sobre la aminofilina en las pruebas de la capacidad vital total y la capacidad respiratoria máxima. La epinefrina se mostró igual, sino superior, a la aminofilina en lo tocante a mejorar los coeficientes, medidos cronológicamente, de la capacidad vital y la corriente espiratoria. En las condiciones de este estudio, no pudo descubrirse ninguna diferencia apreciable entre las dos drogas en lo relativo a duración de la actividad broncodilatadora. Los efectos colaterales observados en este estudio fueron mínimos, pero un poco más frecuentes con la aminofilina por vía venosa.

Resume

Evaluation comparée de l'adrénaline et de l'aminophylline comme agents bronchodilatateurs

On a utilisé les déterminations spirométriques en série pour l'évaluation objective de l'activité bronchodilatatrice de l'adrénaline sous-cutanée et de l'aminophylline intraveineuse.

L'adrénaline sous-cutanée s'est montrée nettement supérieure à l'aminophylline dans les tests de la capacité vitale globale et de la capacité respiratoire maxima. L'adrénaline s'est montrée égale, sinon supérieure, à l'aminophylline pour améliorer les capacités vitales chronométrées et les taux du débit expiratoire. Dans les conditions de l'étude, aucune différence entre les deux drogues, quant à la durée de l'activité dilatatrice, n'a été décelée. Les effets secondaires rencontrés au cours de l'étude étaient minimes; ils étaient un peu plus fréquents avec l'aminophylline intraveineuse.

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THE INDUCTION OF RESPIRATORY ACIDOSIS BY OXYGEN BREATHING[1, 2, 3]

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INTRODUCTION

Attention in recent years has been drawn to the condition of respiratory acidosis in emphysematous patients as precipitated by respiratory infection (1), anesthesia (2), narcotics and sedatives (3), or by administration of high concentrations of oxygen (4, 5). The latter situation has been stressed so frequently that many physicians hesitate to administer oxygen to a dyspneic, emphysematous subject. To combat this therapeutic paradox, various regimens have come into use. The common practice in hospitals is to administer oxygen by mask or nasal catheter for twenty to thirty minutes, followed by breathing of room air for a similar length of time (6). Also, oxygen has been recommended at flows of 1 to 2 L. per min. to be increased after a certain length of time has elapsed for "adaptation" (7). Others believe that lower concentrations of oxygen (30 to 40 per cent) can be administered to relieve anoxia without producing respiratory acidosis (8). The ideal way to administer oxygen safely under such circumstances is with the assistance of a mechanical aid to ventilation (1, 4, 9).

In most of the previous work on respiratory acidosis, little has been done to assess the level of impairment of pulmonary ventilation, below or above which respiratory acidosis may or may not be precipitated by high concentrations of oxygen. It is common clinical experience that such a situation usually is encountered only among the most severe respiratory cripples who are very hypoxic, hypercapneic, and in right heart failure (4, 10). If such a level of respiratory impairment could be defined by simple and readily available tests of pulmonary ventilation, this might be of help in the long-term management of respiratory cripples in relation to oxygen therapy.

A routine procedure in this laboratory for the past several years in studying emphysematous subjects has been to observe the effects of high concentrations of oxygen on both the arterial oxygen saturation as part of a broader study on pulmonary circulation (11) and arterial pH. Although the oxygen was adminis-

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[3] Presented, in part, before the American Federation of Biological Sciences, Atlantic City, New Jersey, April, 1956.

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tered for only short periods of time (six to ten minutes), respiratory acidosis developed in about 18 patients. The purpose of this study is to correlate the appearance of this condition with the degree of impairment of pulmonary ventilation as defined by what is generally considered the most simple and universally available tests of pulmonary ventilation; that is, the maximal breathing capacity and the three-second timed vital capacity. Also, ancillary data will be presented which bear directly on the pathogenesis of respiratory acidosis.

Material and Methods

All of the 209 subjects were exposed to ambient room air and to 99.5 per cent oxygen breathing for seven to ten minutes, during which time various measurements (viz., infra) were made in the postabsorptive basal state. The criteria for normal predicted values for this laboratory have previously been reported (12). Standard laboratory procedures were employed (13–17).

A correction factor of 0.01 pH units for each degree Centigrade of difference between room temperature and body temperature of 37°C. was used. The carbon dioxide and oxygen tensions of arterial blood were determined only on blood drawn while the subject was breathing room air. All measures of ventilation were made with the open-circuit technique and are reported as L. per min. per M^2 of body surface area corrected to standard temperature and pressure, dry.

Minute ventilation during room air breathing was measured during the last three minutes of a seven-minute run; on the other hand, ventilation during the breathing of 99.5 per cent oxygen was measured concomitantly during the entire seven minutes of the open-circuit nitrogen washout technique.

The criterion for respiratory acidosis while breathing high oxygen mixtures was a fall in pH below 7.38. This limit was chosen in preference to 7.40 or 7.39 because it did not occur except in one instance among the relatively normal subjects. If a pH below 7.40 or 7.39 had been used as indicative of respiratory acidosis, almost 10 per cent of the subjects with no impairment or minimal to moderate degree of pulmonary function impairment would have been included.

Respiratory acidosis hereafter refers exclusively to a fall in pH below 7.38 while breathing 99.5 per cent oxygen. Many of the 17 patients who developed respiratory acidosis (acidotics) on oxygen had normal pH values while breathing room air.

Results

The incidence of respiratory acidosis on 99.5 per cent oxygen according to the per cent predicted maximal breathing capacity and three-second timed vital capacity is plotted in figure 1 for the 209 patients studied. There was a sharp breaking point at a timed vital capacity below 50 per cent and a maximal breathing capacity below 40 per cent of predicted. Although there were only 17 patients who developed respiratory acidosis of the 209 patients being studied,[6] the correlation may be observed that, as the degree of impairment in pulmonary ventilation increased, the percentage of subjects who developed respiratory acidosis while breathing 99.5 per cent oxygen increased. For instance, when patients with very low maximal breathing capacities or timed vital capacities of less than 20 per cent of predicted were studied, the incidence of respiratory acidosis was 33 and 50 per cent, respectively.

[6] The one subject who developed respiratory acidosis with normal pulmonary function has been excluded from the 17 other subjects in the subsequent analyses.

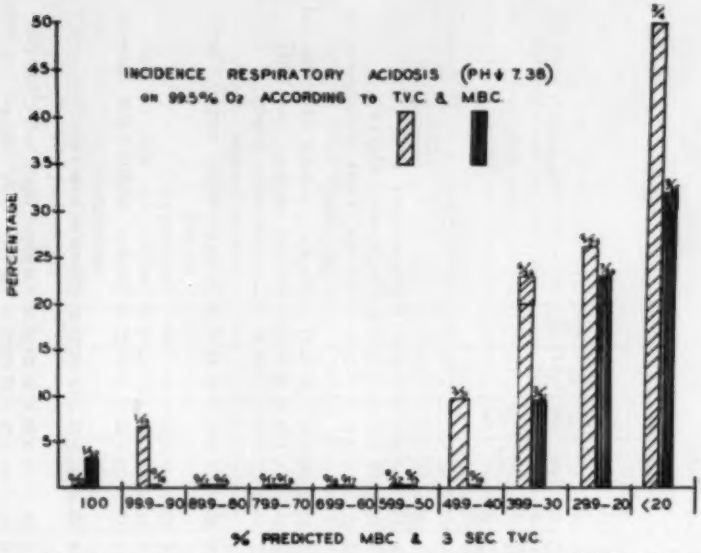

FIG. 1. The percentage of patients who developed respiratory acidosis arranged according to per cent predicted maximal breathing capacity (MBC) and three-second timed vital capacity (TVC). The denominator of the fraction at the top of each bar is the number of subjects with MBC's or TVC's in the range indicated on the abscissa. The numerator is the number of subjects who developed a pH below 7.38 on 99.5 per cent oxygen. The percentage or incidence of acidosis in each group is calculated from this fraction. A total of 209 subjects were studied.

In the subsequent analyses of these data, a comparison is made of the differences (as defined by various tests) between the 17 patients who developed respiratory acidosis on oxygen breathing and the 59 others, as shown in figure 1, with maximal breathing capacities below 40 per cent predicted who did not, although both were exposed to 99.5 per cent oxygen for the same length of time.

The pertinent information on the 17 patients who developed respiratory acidosis on 99.5 per cent oxygen is listed in table 1.

Among the 59 nonacidotic patients, only the mean values of whose data are shown in table I*A*, emphysema was present in 57, accompanied by fibrosis in 17, by cor pulmonale in 15, and by other forms of heart disease in 7. Other disease processes present were asthma (6 cases), pneumoconiosis (4 cases), bronchiectasis (4 cases), pulmonary tuberculosis (6 cases), and pulmonary cysts (one case). Miscellaneous conditions present were Hand-Schüller-Christian disease, cystic fibrosis of the pancreas, and pectum excavatum.

The differences referred to subsequently between the 17 patients who developed respiratory acidosis on 99. 5 per cent oxygen breathing (acidotics) with the 59 who did not (nonacidotics) are statistically significant, the P values for these comparisons being less than 0.05.

TABLE 1

ARTERIAL BLOOD AND PULMONARY FUNCTION* DATA WITH DIAGNOSES ON 17 PATIENTS WHO DEVELOPED RESPIRATORY ACIDOSIS ON 99.5 PER CENT OXYGEN BREATHING

(All with Maximal Breathing Capacity Below 40 Per Cent Predicted)

Case Number	Age	Height	Weight	BSA M^2	Maximal Breathing Capacity Per Cent Predicted	Arterial Blood					Minute Ventilation L/min/M^2		Arterial Saturation %		Three-Second Timed Vital Capacity Per Cent Predicted	Vital Capacity Per Cent Predicted	Total Lung Volume Per Cent Predicted	Per Cent Residual of Total Lung Volume	Alv. N_2 @ 7 Min. O_2	Ventilation Factor	Diagnosis†
						pO_2	pCO_2	CO_2 Content	pH Air	pH 99.5%O_2	Air	99.5%O_2	Air	99.5%O_2							
	Years	*cm.*	*Kg*			*mm. Hg*	*mm. Hg*	*Vol. %*													
991	50M	179	63	1.80	20.8	81.6	40.7	49.1	7.41	7.35	3.98	4.08	87.8	100.0	33.1	45.0	92.8	61.5	3.40	31.5	Emphysema
1010	54M	183	75	1.97	20.7	69.5	46.8	50.7	7.38	7.35	3.01	2.91	91.6	98.5	32.8	42.5	59.2	44.6	2.12	36.5	Emphysema, fibrosis
1129	48M	175	62	1.76	37.2	88.9	45.7	57.1	7.39	7.37	3.41	3.50	93.5	99.2	27.5	27.5	35.6	45.2	1.25	40.0	Asb-dia. earth pneumocon. emphy., fibr.
1113	52F	158	57	1.57	26.7	69.5	46.0	52.7	7.43	7.36	3.04	2.96	87.0	98.4	43.7	50.9	84.9	57.7	6.74	37.9	Emphy., asthma, fibr., cor pul.
786	63M	172	60	1.75	20.8	84.8	42.9	44.3	7.40	7.37	4.45	4.74	91.9	100.0	31.3	41.7	74.1	54.6	2.01	42.1	Bullous emphy., cyst RLL
993	47F	158	65	1.66	25.5	87.5	47.1	41.8	7.58	7.37	5.54	5.38	85.0	91.0	48.7	59.3	104.8	56.2	2.39	39.6	Bronchiectasis, emphy., asthma, cor pul.
835	60M	177	57	1.71	26.1	—	—	40.7	7.40	7.35	4.46	3.94	94.1	99.5	43.0	65.0	114.0	56.1	8.41	37.9	Bronchiectasis, emphy., poly., cor pul.
1011	67M	177	52	1.64	32.1	59.7	49.3	55.3	7.45	7.37	3.58	3.46	87.3	97.4	33.9	55.1	90.3	62.1	9.17	38.1	Emphysema
1200	51M	175	56	1.68	20.7	43.3	75.4	73.5	7.37	7.30	3.92	3.02	73.9	95.5	33.9	61.9	82.6	50.2	17.42	34.8	Emphy., sil., fibr., cor pul.
1164	50M	179	90	2.09	15.1	77.4	49.2	56.0	7.42	7.36	4.29	3.90	90.3	98.5	25.1	43.0	90.4	66.3	3.83	37.9	Emphy., ASHD & EH, congenital failure
1044	58M	179	64	1.81	10.6	51.9	59.9	67.9	7.40	7.35	3.25	3.09	64.5	98.3	19.3	35.8	90.3	73.4	9.91	21.3	Phosgene exposure, emphysema
989	60M	173	56	1.67	12.8	40.4	72.0	68.5	7.33	7.23	2.71	2.87	64.5	100.0	18.7	34.7	79.5	66.6	10.69	23.0	Emphy., fibr., poly., cor pul.
1198	52M	174	57	1.69	16.4	56.1	49.9	56.7	7.43	7.37	3.70	2.51	85.0	94.7	27.8	53.5	100.4	56.1	16.09	29.6	Emphy., poly., cor pul.

1005	56F	171	69	1.81	13.6	39.9	64.0	63.7	7.37	7.29	3.41	3.02	62.3	100.0	24.5	53.6	86.4	59.2	6.38	26.8	Emphy., fibr., poly., cor pul.
1002	41M	189	62	1.86	17.3	50.2	61.0	49.2	7.46	7.35	3.91	3.57	86.5	98.8	27.6	48.9	78.6	59.8	5.30	28.9	Emphy., RHD $\bar{c}$ MS, congenital failure
1214	50M	179	78	1.97	16.4	62.4	66.4	69.5	7.40	7.34	2.75	2.25	85.7	95.1	30.1	59.3	97.6	52.5	13.51	31.4	Pulmonary tuberculosis, fibr., emphy., cyst RLL, cor pul.
1190	50M	164	56	1.62	8.1	48.4	68.1	—	7.39	7.32	3.47	2.06	69.0	95.3	21.0	41.9	64.3	47.1	8.68	27.4	Asthma, emphy., fibr. hypothyroid
Mean $\bar{x}$					20.1	63.2	55.3	56.0	7.41	7.34	3.70	3.37	82.4	97.7	30.7	48.2	83.9	57.0	7.49	33.2	
S.D.‡					7.7	17.1	11.2	3.6	0.05	0.04	0.72	0.86	10.92	2.52	8.45	10.3	18.8	10.5	5.25	6.3	

TABLE 1*A*

MEAN ARTERIAL BLOOD AND PULMONARY FUNCTION DATA ON THE 59 PATIENTS WHO DID NOT DEVELOP RESPIRATORY ACIDOSIS ON 99.5 PER CENT OXYGEN BREATHING

(All With Maximal Breathing Capacity Below 40 Per Cent Predicted)

Mean	25.3	75.0	47.0	52.2	7.45	7.42	3.84	3.86	89.0	98.4	36.7	58.7	97.3	55.9	4.99	37.1		
S.D.‡	7.9	12.7	7.6	8.2	0.03	0.00	0.71	0.64	5.3	1.1	11.0	13.5	21.6	11.5	2.85	8.2		

* Abbreviations of functions are as follows: BSA—body surface area in square meters; pO_2—arterial tension of oxygen; pCO_2—arterial tension of carbon dioxide; CO_2 Content—total carbon dioxide content of arterial blood; pH of arterial blood; per cent resid. of TLV—per cent of total lung volume occupied by the residual volume; ALV. N. $\bar{p}$ 7 min. O_2—alveolar nitrogen after 7 minutes of oxygen breathing.

† Abbreviation of diagnoses are as follows: Emphy.—emphysema; fibr.—fibrosis; poly.—polycythemia; sil.—silicosis; cor pul.—cor pulmonale; RHD $\bar{c}$ MS—rheumatic heart disease with mitral stenosis; cyst.—cystic; EH—enlarged heart; RLL—right lower lobe; Dia. earth pneumocon.—diatomaceous earth pneumoconiosis; ASHD—arteriosclerotic heart disease.

‡ S.D. Standard deviation about the mean.

Arterial blood studies: Considering the arterial blood studies and minute ventilation first, the 17 subjects who developed respiratory acidosis compared with 59 who did not were more hypoxic (pO_2, 63.2; mm. of mercury, vs. 75), more hypercapneic (pCO_2, 55.3; mm. of mercury, vs. 47.0), had a higher total carbon dioxide content (56.0 volume per cent vs. 52.2), and a greater degree of arterial desaturation (82.4 per cent vs. 89.0 per cent) on breathing room air. There was, however, no difference in the minute ventilation or pH on air breathing between the two groups. When the 99.5 per cent oxygen was substituted for room air, a marked fall in the pH occurred in the 17 subjects (7.41 to 7.34) accompanied by a fall in the minute ventilation (3.70 L. per min. per M^2 to 3.37). In contrast the 59 nonacidotics as a group had no significant change in either pH or minute ventilation. Although not shown in any of the tables, the carbon dioxide content increased significantly from 56.0 volume per cent to 57.2 in the group which developed acidosis, but showed no change in the 59 subjects who did not develop acidosis. This small increase in the total carbon dioxide content which occurred in such a short period of time was due entirely to an increase in the pCO_2.

Lung volumes and ventilatory function: Considering now the lung volume and ventilatory data, the 17 subjects who developed respiratory acidosis on 99.5 per cent oxygen, compared with the 59 who did not, had a greater reduction in their maximal breathing capacity (20.1 per cent vs. 25.3 per cent), had a greater impairment in the effective utilization of their vital capacity (three-second timed vital capacity, 30.7 per cent of predicted vs. 36.7 per cent) and, although not shown here, had the same degree of relief of bronchospasm after the administration of an aerosol of isopropyarterenol (Isuprel®). The severe reduction in vital capacity among the 17 subjects who developed respiratory acidosis was the most striking difference between the two groups (vital capacity, 48.2 per cent of predicted vs. 58.7 per cent). This was further manifested by a reduction in the total lung volume (83.9 per cent of predicted vs. 97.3 per cent). Presumably the great reduction in vital capacity and total lung volume of the 17 subjects took place because of fibrosis and/or pulmonary congestion. The degree of emphysema as measured by the per cent residual of the total lung volume is no different for the two groups (57.0 per cent vs. 55.9 per cent); and, statistically speaking, there was no difference in the inequality of the distribution of air in the lungs as shown by the alveolar nitrogen after seven minutes of oxygen breathing for the two groups (7.49 per cent vs. 4.99 per cent). The ventilation factor, however, which takes into account the maximal breathing capacity, three-second timed vital capacity, and per cent residual of total lung volume was lower for the group which developed respiratory acidosis (33.2 vs. 37.1).

Discussion

It has been known for many years that inspiratory mixtures of high oxygen concentration in cardiac and respiratory cripples may result in a reduction of minute ventilation of the magnitude of 20 to 35 per cent (18). Barach and Woodwill (19) and later Richards and Barach (20) noted the rise in carbon dioxide content that accompanied this reduction in ventilation while breathing high

oxygen mixtures of approximately 50 per cent concentration. In very few instances was any lowering of arterial pH into the acidotic ranges observed over long periods of time.

After the role of the effect of hypoxia on the respiratory center as mediated by the carotid and aortic bodies was clarified by Heymans and co-workers (21) and Marshall and Rosenfeld (22), the hazards of high oxygen mixtures in chronic pulmonary disease became known (4, 23, 24). On the basis of clinical experience, coma and acidosis resulting from such therapy are not too common; but when they do occur, a profound therapeutic paradox and medical emergency are encountered. The problems of transposing the data presented in this paper to the clinical situation are many. Perhaps, however, with some qualifying remarks, these difficulties can be resolved. If the per cent predicted maximal breathing capacity and timed vital capacity of a given emphysematous subject were known prior to the administration of oxygen, the potential hazard of inducing respiratory acidosis might be predicted with some degree of accuracy (on the basis of the data in figure 1). Actually, if a pH meter were available, the most direct approach to judging the hazards of oxygen breathing would be to measure the arterial pH directly during oxygen therapy as was done in this experiment.

Granted that a fall in pH of the magnitude observed in some of the patients who developed respiratory acidosis is not great; but with the realization that such a tendency exists, the potential hazard of more severe respiratory acidosis and the development of carbon dioxide narcosis becomes a real problem if respiratory function is further depressed by infection, barbiturates, narcotics, and the administration of anesthesia without adequate ventilation.

The period of oxygen administration in the present experiment was of necessity very short but was of sufficient length to induce respiratory acidosis. Perhaps the incidence of respiratory acidosis would have been greater had longer periods been used, as employed by Wilson and associates (3) and Taquini and co-workers (24) (twenty minutes). However, minute by minute observation in one patient who developed respiratory acidosis revealed that the greatest fall in ventilation and pH took place after three minutes of oxygen breathing, a plateau being reached in both ventilation and pH thereafter. Since acidosis can develop in such a short time, as shown by these studies, and coma can intervene within ten minutes, as observed from previous experience (9), it would seem wise to observe a severe respiratory cripple carefully for a short period of time after commencing oxygen therapy.

Respiratory center in emphysema: Ever since the work of Heymans and associates (21) on the carotid and aortic bodies, many papers on this phase of regulation of the respiratory center have been written (25, 26). The current concept, however, regarding the status of the respiratory center in *some* emphysematous subjects is that this center is not as responsive as a normal respiratory center to the carbon dioxide hydrogen ion stimulus (27–29). Furthermore, it depends on chronic hypoxia (via the carotid and aortic bodies) for its main stimulus (4, 24). If the hypoxic drive is abolished, alveolar ventilation decreases to such an extent that carbon dioxide retention occurs and pH falls. Ordinarily, such

changes should bring about a homeostatic regulatory mechanism to prevent further decrease in ventilation by stimulation directly on the respiratory center, but they fail to do so in this instance.

The reason for the respiratory center's being damaged in emphysema is thought by some to be due to chronic hypercapnia and to be a permanent change (8, 30). The data of Fishman, Samet, and Cournand (8) bring out the point that patients with chronic hypercapnia accompanying emphysema have a greater diminished ventilatory response to high inspired mixtures of carbon dioxide than emphysematous patients without hypercapnia.

The present data, in a general way, conform to established concepts. If it is assumed that the 17 patients who developed respiratory acidosis on oxygen breathing in this study had unresponsive respiratory centers (in that minute ventilation decreased as the carbon dioxide-hydrogen ion stimulus increased), it certainly was true that, as a group, they had more severe hypercapnia than the 59 patients who did not develop respiratory acidosis.

Effect of 30 to 40 per cent oxygen mixtures: On a theoretical ground, any inspiratory mixture of oxygen that relieves hypoxia and restores the arterial tension to approximately 95 mm. or more of mercury should obliterate or ameliorate the ventilatory drive of hypoxia and produce a decrease in alveolar ventilation in subjects dependent on the hypoxic stimulus for respiration. The few data listed in table 2 bring out some interesting points.

It was noted in these 3 subjects that inspiratory mixtures of 30 to 40 per cent oxygen produced respiratory acidosis in 2 cases and a reduction of pH to 7.39 in the third, but not to the extent that 100 per cent oxygen did in all 3 patients. Although the pO_2 is only measured in one instance while breathing 30 to 40 per cent oxygen (and found to be within the physiologic range), there is no reason to assume that a 30 to 40 per cent oxygen mixture which provides an inspiratory pO_2 of 220 to 280 mm. of mercury should not raise the pO_2 to 95 to 100 mm. of mercury. At any rate, it may be assumed that the oxygen concentration on these mixtures, i.e., 30 to 50 per cent, is adequate to subdue or obliterate the hypoxic stimulus to respiration, since the saturation rises to nearly normal levels. The question then arises, why should an inspired oxygen of 100 per cent be responsible for a greater diminution of pH than a 30 to 40 per cent mixture?

The figures given in table 2 for minute ventilation while breathing 30 to 40 per cent mixtures do not reveal greater depression of minute ventilation in all instances; but, if it is assumed for the moment that, in the face of a changing respiratory quotient, the absolute carbon dioxide production reflects more accurately smaller changes in alveolar ventilation than minute ventilation does, then it may be assumed from these data that 99.5 per cent oxygen produces a greater reduction in pH because it reduces alveolar ventilation to a greater extent. Such reasoning would imply that a further mechanism is evoked than just relief of the hypoxic stimulus as mediated via the carotid and aortic bodies to the respiratory center.

To explore these mechanisms, consideration has been given to possibilities of enzyme inhibition (31, 32), further increase in carbon dioxide hydrogen ion

TABLE 2

PRODUCTION OF RESPIRATORY ACIDOSIS WITH 30 TO 40 PER CENT OXYGEN MIXTURES

Case	Diagnosis	Per Cent Predicted Maximal Breathing Capacity	Inspired Air Per Cent Oxygen	Arterial Blood				Minute Ventilation	CO_2 Production	Oxygen Consumption	Respiratory Quotient
				pH	pCO_2 mm. Hg.	pO_2 mm. Hg.	Arterial Saturation Per Cent				
								L/min/M²	*cc/min/M²*	*cc/min/M²*	
No. 1304	Emphysema, fibrosis	27.7	20.8 (air)	7.38	64.8	47.4	79.8	3.49	97	124	0.778
			32.1	7.36	76.1	106.7	91.0	2.77	89	171	0.524
			42.9	7.36	—	—	95.0	2.96	—	—	—
			99.5	7.29	—	—	100.0	2.59	—	—	—
No. 989	Emphysema, fibrosis, polycythemia, cor pulmonale	12.8	20.8 (air)	7.33	72.0	40.4	64.5	2.71	103	128	0.865
			39.7	7.26	—	—	96.0	2.37	88	170	0.514
			99.5	7.23	—	—	100.0	2.87	60	—	—
No. 1344	Fibrothorax, emphysema, fibrosis, cor pulmonale	34.1	20.8 (air)	7.44	45.9	65.3	79.8	5.65	136	183	0.746
			32.1	7.39	—	—	93.6	4.65	122	239	0.512
			99.5	7.36	—	—	100.0	4.65	118	—	—

stimulus with loss of the "dual function" of hemoglobin (33), plus a possible reduction in cerebral blood flow on 100 per cent oxygen (34–36). These mechanisms could very well exert a profound influence on the respiratory center to account for the apparent differences of 100 per cent oxygen and 30 to 40 per cent oxygen on arterial pH listed in table 2.

At any rate, the work of Barach (23) and the present data suggest that inspiratory mixtures of 30 to 40 per cent oxygen do not produce the same degree of respiratory depression in some emphysema patients as pure oxygen does, but do relieve hypoxia. It seems inadvisable to administer extremely high inspiratory concentrations of oxygen for the relief of hypoxia in patients with severe emphysema prone to respiratory acidosis. Even if artificial means of ventilation are available, the inspiratory concentration of oxygen should be reduced to approximately 40 per cent.

SUMMARY

Two hundred and nine patients with virtually no impairment to severe impairment of pulmonary function were subjected to short periods (six to ten minutes) of breathing pure oxygen (99.5 per cent). An attempt was made to correlate the appearance of respiratory acidosis (a fall in arterial pH below 7.38 while breathing 99.5 per cent oxygen) with the degree of impairment of pulmonary ventilation as measured by the maximal breathing capacity and the three-second timed vital capacity.

Respiratory acidosis (as defined above) was observed in 18 of the 209 patients studied. With the exception of one normal subject, respiratory acidosis appeared only in those patients who had maximal breathing capacities and three-second timed vital capacities below 40 to 50 per cent, respectively, of their predicted values. The incidence rose markedly as patients with the most severe impairment were tested.

Pulmonary fibrosis and/or congestion was common among the 17 subjects who developed respiratory acidosis on oxygen breathing. Data on 3 cases of emphysema are presented in which respiratory acidosis of a lesser degree was produced and hypoxia was relieved by inspiratory mixtures of 30 to 40 per cent oxygen rather than by a mixture of 99.5 per cent.

Sumario

La Inducción de Acidosis Respiratoria por la Aspiración de Oxígeno

A 209 enfermos cuya función pulmonar variaba de virtualmente inafectada a gravemente afectada se les sometió durante breves períodos de tiempo (seis a diez minutos) a la respiración de oxígeno puro (99.5 por ciento). Tratóse de correlacionar la aparición de acidosis respiratoria (baja del pH arterial a menos de 7.38 mientras se respiraba oxígeno de 99.5 por ciento) con el grado de insuficiencia de la aireación pulmonar, medida esta por la capacidad respiratoria máxima y la capacidad vital determinada en tres segundos.

Se observó acidosis respiratoria (en la forma definida) en 18 de los 209 enfermos estudiados. Exceptuado un sujeto normal, no apareció acidosis respiratoria más que en enfermos que tenían capacidades respiratorias máximas y capacidades vitales de tres segundos inferiores en 40 a 50 por ciento, respectivamente, a los valores predichos. La incidencia se elevó decididamente al comprobar a los enfermos que tenían la insuficiencia en forma más grave.

La fibrosis y/o la congestión pulmonares fueron comunes en los 17 enfermos que manifestaron acidosis respiratoria al respirar oxígeno puro. Se presentan datos relativos a 3 casos de enfisema en los que se produjo acidosis respiratoria de menor intensidad y en que se alivió la hipoxia aspirando mezclas de 30 a 40 por ciento de oxígeno más bien que una mezcla de 99.5 por ciento.

Resume

Induction de l'acidose respiratoire par inhalation d'oxygène

Deux cent-neuf malades, chez lesquels le degré de la fonction pulmonaire observée variait entre le déficit pratiquement nul et l'altération marquée, ont été soumis à l'inhalation d'oxygène pur (99,5%) pendant de courtes périodes (6 à 10 minutes). Les auteurs ont tenté d'établir une corrélation entre la survenue de l'acidose respiratoire (c.a.d. la chute du pH artériel au-dessous de 7,38 au cours de l'inhalation d'oxygène à 99,5%) et le degré de réduction de la ventilation pulmonaire déterminé par la capacité respiratoire maxima et par la capacité vitale chronométrée pendant trois secondes.

L'acidose respiratoire (telle qu'elle est définie ci-dessus) fut observée ches 18 des 209 patients étudiés. A l'exception d'un sujet normal, l'acidose respiratoire n'est apparue que chez les malades dont la capacité respiratoire maxima et la capacité vitale déterminée pendant une période de trois secondes étaient respectivement réduites de 40 et de 50% par rapport aux valeurs prévisibles. L'incidence s'est nettement élevée dans la mesure où, dans les cas testés, l'altération de la fonction respiratoire était la plus marquée.

La sclérose pulmonaire, avec ou sans état congestif, était fréquente parmi les 17 sujets chez lesquels l'acidose respiratoire se produisait lors de l'inhalation d'oxygène. Les données obtenues dans 3 cas d'emphysème, où une acidose respiratoire de degré moins marqué

avec hypoxémie a été supprimée par l'inhalation de mélanges d'oxygène à 30 et 40% plutôt que par le mélange à 99,5%, sont présentées.

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HISTOPLASMOSIS[1]

A Review of One Hundred Consecutively Hospitalized Patients

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Introduction

Until about fifteen years ago, histoplasmosis was considered to be an acute, progressive, and highly fatal disease, which was known mainly to a few physicians and mycologists working in endemic areas. Since 1940 it has been recognized that the most common type of infection is benign, self-limiting, and apparently asymptomatic; and detected only by the demonstration of skin sensitivity. Between these two extremes, however, a large number of patients are found with varied manifestations. The recent finding (1) of 114 proved cases of chronic progressive (cavitary) histoplasmosis, in tuberculosis hospitals in the central endemic zone (U. S. Midwest), as incorrectly diagnosed as tuberculosis despite sputum negative for tubercle bacilli, dramatically indicates the need for a more comprehensive understanding of this disease.

With the large migrations since 1940 of our population into, through, and from the highly endemic areas, the problem is no longer confined to any one region. There has been a widespread scattering of the disease, so that histoplasmosis must be considered in the differential diagnosis of pulmonary lesions, especially chronic lesions, in nonendemic as well as in endemic areas.

The present report consists of an analysis of the findings in 100 patients whose final diagnosis was histoplasmosis and who were consecutively hospitalized at Fitzsimons Army Hospital from June, 1951, to September, 1955 (table 1).

Observations

It must be emphasized that these patients represent a selective group in that they are derived from a selected segment of the population. Military personnel are predominantly male, the majority are in the eighteen- to forty-five-year age group, and the probability of their having been exposed to dust in one or more endemic areas is greater than would be expected in a comparably aged group from the general population. Furthermore, it must be noted that all of these patients were referred to Fitzsimons, a pulmonary disease center, because there were demonstrable lesions in the chest roentgenograms. The diagnosis on referral in the vast majority of cases was pulmonary tuberculosis, and the remaining cases represented diagnostic problems usually designated as "pulmonary inflammatory disease of undetermined etiology and possible bronchogenic carcinoma." Surprisingly, in only one case was histoplasmosis diagnosed or suspected prior to admission to this hospital.

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The diagnosis of histoplasmosis was documented in 65 patients: by isolating the fungus in the sputum and gastric and bronchial washings from one patient; by culturing the organism from the resected pulmonary tissues of 3 patients; by demonstration of the organism in the resected pulmonary lesions of 64 patients. The remaining 35 diagnoses were presumptive and were based upon: (*1*) a history of having been in an endemic area, a positive reaction to the histoplasmin skin test, and diagnostically positive serologic tests in 13 patients; (*2*) a history of having been in an endemic area, a positive reaction to the histoplasmin skin test, a negative reaction to second-strength PPD and coccidioidin skin tests in 22 patients.

General Characteristics of the Group

Because of the nature of the hospital, there was naturally a predominance of males, 82 as opposed to only 18 females. The range of age in the group was from ten to sixty-three years, 84 patients being between twenty and forty years of age. The youngest was a ten-year-old female dependent; the eldest, a sixty-three-year old male. There were 99 whites and one Negro in the group. Since no racial predisposition for histoplasmosis has been described and since the usual distribution of the patient population is about 3 whites to one Negro, this may be a significant observation (table 2).

TABLE 1

100 CASES OF HISTOPLASMOSIS

Diagnosis	
1. Documented	65
a. Resected pulmonary tissue	64
b. Sputum, gastric and bronchial washings	1
2. Presumptive	35
a. Area, skin, and serologic tests positive	13
b. Area, histoplasmin positive; second-strength PPD and coccidioidin negative	22

TABLE 2

100 CASES OF HISTOPLASMOSIS

Sex:	Male	82
	Female	18
Race:	White	99
	Negro	1
Age:	Less than 20 years	4
	20–30 years	55
	31–40 years	29
	41–50 years	9
	51–60 years	2
	61–70 years	1

Youngest, 10-year-old female
Oldest, 63-year-old male
84% were between the age of 20–40 years

Ninety-four of the patients in this series gave a history of having been in one or more endemic areas. Seventy-six of them had been in the central endemic zone of the Mississippi, Missouri, and Ohio River region: the majority, especially those with symptoms in the past, had been stationed in Missouri, eastern Kansas, or Louisiana. Twenty-five patients gave a history of having been in the southeastern states, principally Virginia. Eighteen had been stationed in the Panama Canal Zone, and 9 had been in North Africa. Some of the 94 had been born and raised in an endemic area, but all had been in an endemic area either recently, prior to, or at the time the pulmonary lesion was detected in the chest roentgenogram. The geographic data recorded from 6 patients were too inadequate to show whether or not they had been in any of the above regions.

Symptoms

The extensive skin testing and roentgenographic surveys in the central endemic zone seem to indicate that at least 90 per cent of the persons infected with *Histoplasma capsulatum* develop a benign, self-limiting form of the disease, which is either asymptomatic or is associated with mild respiratory symptoms. Acute or chronic active disease may be accompanied by symptoms varying from a mild respiratory tract infection to a severe, generalized, systemic illness which may terminate fatally. Residual lesions are more likely to produce symptoms related to local pressure or partial bronchial obstruction, if they produce symptoms at all.

In the present series, 54 patients were symptomatic prior to or at the time of entering this hospital. Cough was reported by 38 patients. The cough was generally nonproductive or only slightly productive. Chest pain occurred in 14 patients. It varied from a dull unilateral ache to sharp, pleuritic pain. Weight loss was noted by 10 patients. It ranged from 8 to 34 pounds, the majority reporting a loss of 20 pounds. Fatigue was noted by 8, malaise by 8, and blood streaking without frank hemoptysis by 4 patients. Spontaneous pneumothorax occurrred in 2 patients (table 3).

The predominant roentgenographic patterns found in the chest films of the 54 patients with symptoms are listed in table 4.

Physical Signs

Physical signs were present in 12 patients. In 2 they were referrable to spontaneous pneumothorax, and in one to pleural effusion, so that only 9 had signs

TABLE 3

100 CASES OF HISTOPLASMOSIS
Symptoms Present in 54 Cases

1. Cough (nonproductive or slightly productive)	38
2. Chest pain (varied from dull ache to pleuritic)	14
3. Weight loss (varied from 8 to 34 pounds)	10
4. Fatigue	8
5. Malaise	8
6. Blood streaking (no frank hemoptysis)	4
7. Spontaneous pneumothorax	2

referrable to parenchymal pulmonary lesions. Three of the patients had signs associated with the right middle lobe syndrome. The remaining 6 patients had a diffuse, pneumonic type of infiltration: 2 of these had cavitary disease, one had bronchiectasis, and one had bronchogenic carcinoma and histoplasmosis in the same segment of the left upper lobe (table 5).

Roentgenograms

Roentgenographic abnormalities were noted in the chest films of all patients in the present series, and the predominant patterns are listed in table 6. The lesions were right-sided in 54, left-sided in 39, and bilateral in 7 patients. The right middle lobe was involved in 15, whereas the remaining lobes were equally affected.

"Coin" lesions: Discrete circumscribed densities sharply demarcated from the surrounding lung and measuring from 1 to 3 cm. in diameter were classified as "coin" lesions. In the present series there were 55 such abnormalities; 13 were associated with hilar adenopathy.

Soft linear and soft nodular infiltrations: Pneumonic and linear infiltrations were found in 32 patients. These were unilateral in 28, bilateral in 4, and associated with hilar adenopathy in 24 patients.

TABLE 4

ROENTGENOGRAPHIC PATTERNS ASSOCIATED WITH 51 SYMPTOMATIC CASES OF HISTOPLASMOSIS

1. Soft linear and nodular infiltration	25
2. "Coin" lesion	21
3. Hilar and mediastinal adenopathy	19
a. Alone	6
b. Associated with parenchymal infiltration	13
4. Cavitation	4
5. Contraction of right middle lobe	4
6. Nodules (5–8 m.m. in diameter)	6
7. Pleurisy with effusion	2
8. Pneumothorax	2

TABLE 5

PHYSICAL SIGNS

Present	12
Absent	88
Types of lesions, associated physical signs	
Diffuse infiltration	6
Right middle lobe syndrome	3
Cavities	2
Spontaneous pneumothorax	2
"Coin" lesion and hilar node	1
Pleural effusion	1
Bronchiectasis	1
Bronchogenic carcinoma	1

All cases with physical signs were symptomatic.

TABLE 6

100 CASES OF HISTOPLASMOSIS

Predominant Roentgenographic Patterns

(1) "Coin"	55
(2) Soft linear and nodular infiltration	32
a. Unilateral	28
b. Bilateral	4
c. Associated hilar adenopathy	24
(3) Hilar mediastinal adenopathy	37
a. Associated with parenchymal lesion	31
(4) Nodules (3–8 mm. in diameter)	15
(5) Cavitation	4
(6) Pleurisy with effusion	2
(7) Pneumothorax	2
(8) Contraction of right middle lobe	5

Nodules: Discrete circumscribed densities sharply demarcated from the surrounding lung and measuring 3 to 8 mm. in diameter were classified as nodules. These were usually multiple, and were found in more than one pulmonary segment or lobe. Nodules when associated with "coin" lesions in the same lobe were not tabulated, but a "coin" lesion in one lobe and a nodule in another were counted separately. There were 7 patients with nodules alone and 8 in whom "coin" and nodular lesions were present in different lobes.

Cavitation: Gross cavitation was associated with upper lobe infiltration in 4 patients. This type of lesion has been reported only in adults; the majority of them had been incorrectly diagnosed and treated as tuberculosis.

Pleurisy with effusion obscuring the costophrenic angle was found in 2 patients.

Pneumothorax: Right-sided spontaneous pneumothorax occurred in 2 patients with nodular disease in the posterior segment of the right upper lobe.

Contracted right middle lobe: Right middle lobe syndrome was found in 5 patients who had contracted right middle lobe, "coin" lesion, and hilar adenopathy.

Hilar adenopathy: Enlarged hilar and paratracheal lymph nodes were noted in conjunction with parenchymal lesions in 31; whereas in 6 they were seen alone.

The roentgenographic lesions found in histoplasmosis are not characteristic or diagnostic, but closely resemble the various patterns seen in other diseases, especially tuberculosis. Furthermore, the acute pneumonic lesions may be indistinguishable from those of any viral or bacterial pneumonia. Hilar and mediastinal lymphadenopathy, alone or in conjunction with parenchymal lesions, must be distinguished from tuberculosis, Hodgkin's disease, sarcoidosis, lymphomas, and mediastinal neoplasms.

"Coin" lesions due to histoplasmosis, coccidioidomycosis, blastomycosis, cryptococcosis, tuberculosis, and neoplasms can be neither definitely differentiated from one another nor definitely diagnosed by the appearance in the chest film. Upon reviewing a large series of cases with "coin" lesions proved to be of mycotic, tuberculous, or neoplastic origin, it was found that the so-called "target"

lesions, with dense central core and less dense surrounding halo, were seen only in those proved to be due to histoplasmosis. It was noted occasionally, however, that the "typical target" appearance was not seen in all films of a series, probably due to slightly different positioning of the patient. It is believed that the "target" lesion is merely suggestive and not diagnostic of histoplasmosis.

Calcification was not seen in this series in the widespread, reticulated, or pneumonic forms of the disease, but was found only in the nodular densities. This compares with the observation of others. It is to be noted that calcification has been observed to occur as early as four months after clinical illness with histoplasmosis (2), but the usual time required is two to three years (3). This is in contrast to tuberculosis, in which the earliest appearance of calcification in the chest film is usually about ten months following the onset of infection (4).

Skin Reaction to Histoplasmin

In the present series, simultaneous skin testing was done with first-strength PPD and coccidioidin and histoplasmin in 1:100 dilutions. The tests were read at twenty-four and forty-eight hours. If the test with first-strength PPD was negative, it was repeated with second-strength PPD. In some, but not all, in whom the reaction to coccidioidin 1:100 was negative, the test was repeated using coccidioidin 1:10.

The tests were performed on 99 patients; 96 reacted positively to histoplasmin. One patient was not tested, and 3 patients failed to react to all skin tests; but in each the organism was identified in the resected lesions. The tuberculin tests were positive in 40 patients, all of whom were in the surgically resected group in which the fungus was identified. No tubercle bacilli were found in the surgically removed tissues by histologic examination, cultures, or animal inoculation. None of the 35 patients of the presumptive group reacted to skin tests with PPD, first or second strength. The coccidioidin skin test was positive in 15 patients, but in none of these was *Coccidioides immitis* identified by histologic study, culture, or animal inoculation.

Serologic Tests for Histoplasmosis

Serologic tests were done on 50 patients in the present series. In 34 of the group, one or more tests were positive. The tests were usually repeated for verification and also to determine rising or falling titers. The tests were all done by Charlotte Campbell, in the Army Mycology Laboratory, Walter Reed Hospital. A complement-fixing antibody titer of 1:16 or greater was considered diagnostic of "serologically proved" histoplasmosis.

Of the 34 patients with positive serologic reactions, 32 had complement-fixing antibodies for histoplasmosis: 22 in a titer of 1:16 or greater (ranging from 1:16 to 1:512), and 4 in a titer of less than 1:8. Complement-fixing antibodies for blastomycosis were found in the sera of 9 patients, and all were considered as cross-reactions: one had a titer of 1:8, the remainder were lower. All serologic tests for coccidioidomycosis were negative.

The collodion agglutination test was positive in 8 of the 50 patients with reported serologic reactions. In 2 patients, whose sera were negative in complement-

fixation tests for all three fungi, the titer was sufficiently high to indicate a fungal infection. The disease was proved in these 2 by identification of the organism in the resected lesions.

Twenty-one of the 34 patients with positive serologic reactions had their lesions removed surgically. In 18 of these patients the organism was identified histologically by special staining techniques alone, and in the remaining 3 the fungus was also isolated by culture.

The predominant roentgenographic patterns found in the chest films of the 34 patients with positive serologic reactions are shown in table 7.

Mycology

Cultures of sputum and of gastric and/or bronchial washings were done on each patient in this series and upon the 66 surgical specimens. One patient with cavitary disease had cultures of sputum and gastric and bronchial washings positive for *H. capsulatum*: in similar specimens from the remaining 99 patients the fungus was not isolated. The organism was cultured from only 3 of the 66 surgical specimens, even though it was readily demonstrated in all by tissue-staining technique. Therefore, histoplasmosis was proved by culture in 4 of 100 patients.

All sputum, gastric and bronchial washings, and surgical specimens were examined closely and cultured for tubercle bacilli. No tubercle bacilli were seen or cultured from any of these specimens.

Treatment

The primary, benign type of infection is a self-limiting disease which needs no treatment, and the epidemic forms have healed without any specific therapy. There are no effective drugs for the more serious forms, as acute and chronic disseminated and chronic progressive histoplasmosis.

The surgical excision of localized pulmonary lesions of histoplasmosis has been performed successfully, with low morbidity and low mortality, and apparently without spread of the disease. The procedure was done originally upon lesions mistakenly diagnosed as tuberculosis, the mycotic nature being recognized only following pathologic examination of the resected tissue.

Surgery was performed on 66 patients in the present series. There was a total of 84 procedures: 58 wedge resections, 11 hilar lymph node excisions, 7 segmental

TABLE 7

TYPES OF LESION ASSOCIATED WITH POSITIVE SEROLOGIC TESTS

"Coin"	13
Infiltrate and hilar node	6
Infiltrate alone	5
Infiltrate and cavitation	4
Hilar node alone	3
Infiltration and effusion	1
"Coin" and hilar node	1
Bilateral calcified nodules	1

resections, 5 lobectomies, 2 decortications, and one pneumonectomy (table 8). The predominant roentgenographic pattern of this group was 44 "coin" lesions, 11 soft nodular and linear infiltrations, 5 right middle lobe syndromes (contracted lobe and enlarged lymph nodes), 2 soft irregular nodules, 2 hilar adenopathy alone, one patient with a large cavity and perifocal infiltration, and one with recurrent pneumothorax.

None of these patients were acutely ill immediately prior to or at the time of surgery, and the chest films revealed localized lesions, so that extensive surgery was not necessary. Pneumonectomy was performed on one patient for bronchogenic carcinoma, however; histoplasmosis was found to be coexistent upon examination of the resected tissue. There were five lobectomies for right middle lobe syndrome: all of the patients had contracted right middle lobes, nodular residual lesions, and hilar adenopathy.

Segmental resection was performed on 7 patients: on 6 for disease limited to one pulmonary segment (2 of whom had persistent cavities) and on one patient for the treatment of postoperative complications following wedge resection of a "coin" lesion.

Single or multiple wedge resections were done for nodules, "coin" lesions, or small infiltrations in one or more pulmonary segments. One patient with a nodular lesion, subpleural blebs, and a recurrent spontaneous pneumothorax was treated by wedge resection with good results. Hilar lymph node excision was usually combined with some other excisional procedure, but in 2 patients this was done alone. Two patients with an associated small pleural effusion had concomitant decortications.

There was only one serious complication in this series. One patient developed a large hematoma at the site of a wedge resection in the posterior basal segment of the left lower lobe. This led to collapse of the basal segments and the accumulation of serosanguineous fluid in the left pleural space. The condition persisted despite vigorous attempts to alleviate it. A basal segmental resection was then performed, following which the patient's recovery was uneventful.

Pathology

The manner in which histoplasmosis is acquired is still open to question, since apparently well-documented cases have been described in which the skin and gastrointestinal tract were the portals of entry. The oral route may be a

TABLE 8

SURGICAL PROCEDURES AND ASSOCIATED LESION

Pneumonectomy	1	(bronchogenic carcinoma and histoplasmosis)
Lobectomy	5	(4 right middle lobe syndrome; 1 "coin")
Segmental resection	7	(2 infiltration and cavitation; 2 infiltration; 1 "coin"; 1 nodule and bronchiectasis; 1 for treatment of surgical complications)
Wedge resection	58	(45 "coin"; 8 infiltration; 1 calcified nodule; 3 soft nodule; 1 nodule and blebs
Hilar node excision	11	(11 hilar adenopathy)
Decortication	2	(2 associated pleural effusion)

major pathway for the infection in infants and very young children. It is generally believed, however, that the lung is the usual pathway, and this appears to be true in all of the patients in the present series.

In the present series, the surgical specimens from 66 patients were examined immediately postoperatively and were cultured for *M. tuberculosis* and fungi. In none of the specimens was *M. tuberculosis* demonstrated by microscopy, culture, animal inoculation, or acid-fast stains of the tissue. *H. capsulatum* was isolated by culture from the resected material of 3 patients, and these findings were confirmed by special staining methods in the entire group of 66 resected specimens. However, upon review of the records and tissue slides by the Armed Forces Institute of Pathology one case was diagnosed: "Granuloma Lung—Probably Due to Histoplasmosis." Consequently, this case has been classified with the group of presumptively diagnosed subjects. The patient had a history of being in an endemic area; the histoplasmin skin test was positive, second-strength PPD repeatedly negative, and a complement-fixation test for Histoplasma was "diagnostically positive." In the remaining 65 patients the diagnosis of the Armed Forces Institute of Pathology and of Puckett at Fitzsimons Army Hospital concurred. The fungus was morphologically demonstrated to the satisfaction of the pathologists of both institutions. The morphologic findings, when viewed in conjunction with the clinical records, were taken as adequate for a diagnosis of histoplasmosis.

Discussion

The present series demonstrates the various types of diagnostic problems which histoplasmosis may offer to the medical service of a general hospital, especially in nonendemic areas, where the diagnosis is relatively rare. Although each patient received a medical evaluation prior to admission to Fitzsimons, in only one was the possibility of histoplasmosis mentioned.

During the past ten years, interest in this disease has increased markedly, especially in the United States, as verified by the continuous increase of cases reported in the world literature: from 71 in 1945 (5) to 139 in 1951 (6), followed by a sharp rise since 1953. Eighty per cent of the reported cases originate in the United States. This does not necessarily imply that the major endemic area of the world is in the Mississippi, Missouri, and Ohio River valleys, but may merely indicate that the intensive survey and research programs in these regions have been responsible for finding more authenticated cases. It should be noted, however, that, as a result of histoplasmin skin testing and chest roentgenographic survey programs, it has been estimated that more than 20 million people in the United States have been infected with *H. capsulatum*.

Several clinical classifications of the disease have been made, none of which is entirely satisfactory. However, some general outline is necessary for an intelligent discussion and understanding of the illness.

Histoplasmosis may be either of a primary or re-infection type:

I. *Primary type:*

1. Acute, symptomatic and progressively fatal histoplasmosis is characterized by fever, anemia, leukopenia, occasionally a leukemoid reaction, weakness, and hepatosplenomeg-

aly. The course may be rapidly progressive, with widespread dissemination and fatal termination, or it may become chronically progressive, with widespread dissemination, lasting for months or years.

2. Acute, symptomatic, nonfatal histoplasmosis may be mild, moderate, or severe. It affects children and adults alike, and is characterized by an influenza-like syndrome, which is frequently incorrectly diagnosed as influenza or primary atypical pneumonia. The onset is sudden, with nonproductive cough, chest pain, and fever. This form has been found to occur sporadically or in epidemics. Recovery is usual, with healing by fibrosis and calcification. Occasionally the disease may develop into the chronic progressive form, and rarely this may go further to generalized dissemination.

3. Acute, asymptomatic, nonfatal histoplasmosis is the most common form of the disease. The course is benign, and healing occurs with either no evidence of pulmonary involvement or with multiple fibrotic and calcified lesions in the lungs and/or the spleen.

II. *Reinfection type:* This type of histoplasmosis has been so called because it resembles the chronic reinfection type of tuberculosis. Roentgenographically the two are indistinguishable. Chronic, progressive, cavitary, and nodular lesions are found in the lungs. The fungus usually can be cultured from fresh sputum specimens, which are persistently negative for tubercle bacilli unless the two diseases are coexistent. The disease may progress to a generalized dissemination and fatal termination.

The primary pulmonary infection begins with implantation of the fungus in the peripheral alveoli, producing a nonspecific reaction similar to that which has been described in tuberculosis. The lesion progresses to a coagulative type of pneumonia, which later becomes encapsulated and contains organisms in the central portion. This may persist as an isolated "round" lesion in the lung, or it may heal by complete resolution. Frequently, if not always, there is involvement of the regional lymph nodes producing a primary complex similar to the Ranke complex of tuberculosis.

The progressive form may develop from the primary without a latent period or it may be due to reinfection. Hilar lymph node involvement is only a minor component of the progressive disease. Spread is always from the mother lesion: peripherally, to involve the pleura producing a stellate pleural plaque; centrally, by the lymphohematogenous route, to produce smaller daughter lesions in the surrounding lung tissue or to involve the regional hilar lymph nodes or, finally, to disseminate widely throughout the entire body.

The chronic disseminating form, arising from either the primary or reinfection type, may involve all systems and organs of the body. This form behaves like sarcoidosis and has been frequently misdiagnosed as such. Death in almost all cases is due to invasion of the adrenals, with the production of adrenal insufficiency.

Enlargement of the mediastinal lymph nodes may be associated with either the primary or reinfection type, with either localized or diffuse pulmonary lesions, or may be the only demonstrable lesion. The latter is difficult to differentiate from Hodgkin's disease, sarcoidosis, and leukemia.

Although most of the patients of the present series were not seen during the early stages of their disease, a few did present, upon admission to this hospital, with an influenza-like syndrome associated with acute histoplasmosis (figures 1, 2, and 3). This compares with the writers' observations in a similar study of

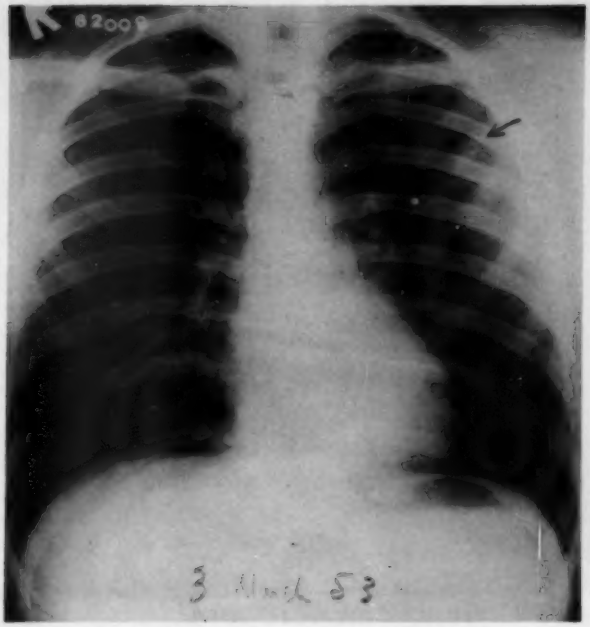

FIG. 1. Chest roentgenogram showing a soft infiltration in the second anterior interspace on the left.

coccidioidomycosis. In a general hospital or pulmonary disease center in a nonendemic area, it is to be expected that the majority of patients would not be seen during the acute stage, but rather with chronic or residual lesions which may be either active or inactive. Progressive or resolving infection may be symptomatic, whereas inactive or well-localized disease is usually silent. However, histoplasmosis heals with marked perifocal fibrosis, much more so than either tuberculosis or coccidioidomycosis, so that, not infrequently, obstructive phenomena with secondary infection develop, producing signs and symptoms.

It is interesting to note that only 9 of the 54 patients with symptoms had physical signs referrable to the pulmonary parenchyma. Moreover, 5 of the patients with symptoms had the right middle lobe syndrome, but only 3 of these had physical findings. Also, of the 5 patients with right middle lobe syndrome, 4 were found in the first 20 patients and only one in the remaining 80 patients. This last observation is worthy of comment since it clearly demonstrates the fallacy of using percentages and drawing universal conclusions from small series.

Roentgenographically there is no typical or diagnostically characteristic pattern. The chest film may simulate any of the lesions seen in patients with tuberculosis, pulmonary mycoses, other granulomas, or intrathoracic neoplasms. Therefore, histoplasmosis must be considered in the differential diagnosis of all

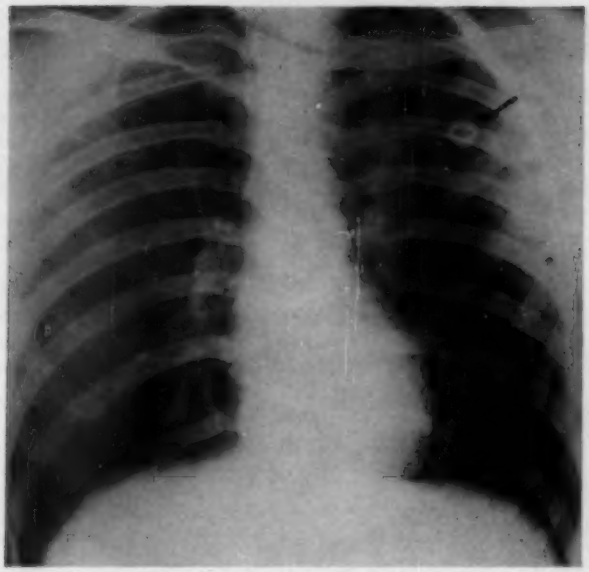

FIG. 2. Chest roentgenogram of patient of figure 1 taken fifteen days later, showing a cavity in the area of the previously seen soft infiltration.

pulmonary lesions in endemic areas, and all chronic pulmonary lesions in non-endemic areas.

It is the writers' experience that the histoplasmin skin test has the same significance as the tuberculin skin test: that is, a positive reaction indicates infection with *H. capsulatum*, past or present. Cross-reactions have been reported with coccidioidin and blastomycin, but always to a lesser degree than with histoplasmin. Furthermore, histoplasmin may cross-react in coccidioidomycosis and blastomycosis, but to a lesser degree than the specific skin test.

There are three serologic tests for histoplasmosis: the precipitin, the histoplasmin collodion agglutination, and the complement-fixation tests. The clinical significance of the precipitin test has not been completely evaluated. However, the collodion agglutination test, once thought to be diagnostically significant, has been found to cross-react so frequently with coccidioidomycosis and blastomycosis that positive reactions are now considered presumptive evidence that an infection is mycotic in origin rather than indicative of histoplasmosis.

The complement-fixation test is so specific, sensitive, and complex that, presently, it is performed by only a few specialized laboratories. Since the diagnostically significant titer varies with the laboratory, interpretation of the test must take this fact into consideration. Campbell, who performed the serologic tests for the present series, has established a titer of 1:16 or greater as diagnostic of "serologically proved" histoplasmosis. Furcolow, however, in his laboratory, considers 1:8 the diagnostically significant level.

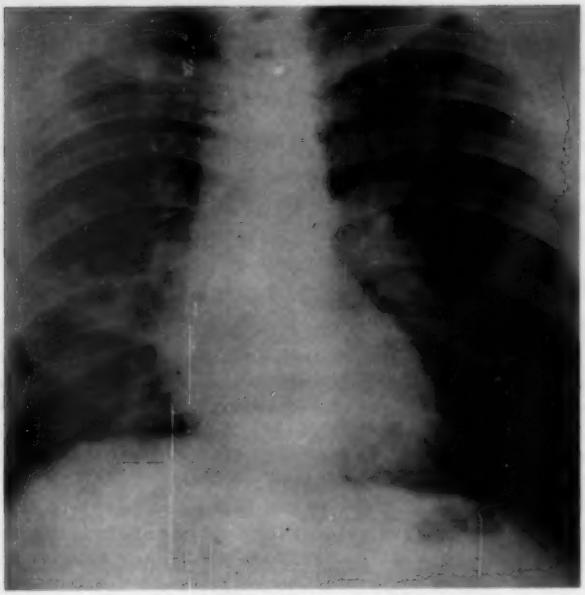

FIG. 3. Chest roentgenogram of patient with proved histoplasmosis. Note the soft infiltration throughout the right lung field and the enlarged hilar lymph nodes.

The majority of patients in this series were admitted to Fitzsimons with the diagnosis of tuberculosis. Although tuberculin, histoplasmin, and coccidioidin skin tests were done simultaneously on all but one patient upon admission, serologic tests for fungi were not done unless mycotic infection was strongly suspected early during hospitalization.

The solitary, discrete, circumscribed pulmonary lesions ("coin" lesions) were removed surgically to establish the diagnosis. During the early part of this study the preoperative diagnosis in these cases was either suspected tuberculosis or possible neoplasm. The meticulous pathologic studies by Puckett established the etiology as histoplasmosis, and excluded tuberculosis, coccidioidomycosis, and neoplasm. In this group serologic tests for fungi were not done.

Bronchogenic carcinoma had been previously diagnosed in one patient referred for surgery. Neither skin nor serologic tests were done in this case even though active histoplasmosis was found in the same lobe as the neoplasm.

It is interesting to note that the surgical excision of localized pulmonary lesions of histoplasmosis has been performed successfully, with low morbidity and low mortality, and apparently without spread of the disease. In the 66 surgical patients there were one pneumonectomy, 5 lobectomies, and 7 segmental resections; the remainder had wedge resections and hilar lymph node excisions. The pneumonectomy was performed for bronchogenic carcinoma, but histoplasmosis was also found to be present. There were 5 right middle lobectomies for "coin" lesions

in contracted right middle lobes, associated with enlarged hilar nodes and symptoms. The segmental resections were for cavitary or soft pneumonic lesions confined to a single segment. The majority of patients had wedge resections of well-localized and stable "coin" lesions or nodules. Enlarged hilar nodes were removed concurrently with other procedures.

Although *H. capsulatum* was demonstrated morphologically in all of the surgical specimens, the organism was recovered by culture from the tissue of only 3 patients and from the sputum and bronchial and gastric washings of one other. If all of the patients in this series had histoplasmosis, why was the organism recovered by culture from only 4? There are two possible answers; and either or both could have been responsible:

1. The delay between collection of the specimen and inoculation of the medium was too great. It is well known that medium inoculated with freshly collected specimens yields a much greater number of positive cultures than medium inoculated with organic material which has been allowed to stand at room temperature for several hours. Although the time interval apparently was too long for *H. capsulatum*, it was not too long for *M. tuberculosis*. Whenever acid-fast bacilli were identified morphologically, they could be cultured using this procedure.
2. The organisms found in the resected tissues may have been in a state of decreased metabolic activity, so that they could not be cultured by the usual methods; or they may have been dead. This could have been an important factor with many of the specimens, since the majority of surgical patients had well-localized and stable lesions.

The morphologic and staining characteristics of *H. capsulatum* in tissues, using the periodic acid-Schiff reagents, are now so well known that Puckett (7) believes a definite diagnosis can be made by this method. Typically, the organisms are round or ovoid, 3 to 5 microns in diameter, and appear intracellularly. However, extracellular organisms have been reported. With the PAS technique they have a well-defined, thin, limiting membrane and a nucleus which stains red. All specimens examined in this series showed the fungus in clusters of four to ten, as well as diffusely scattered single organisms. It should be noted that variations in the preparation of the staining reagents will produce variations in the quality of the stain produced. Thus, for consistent and reproducible results a standard method of preparation should be used.

Summary

An analysis of the finding in 100 consecutively hospitalized patients with the final diagnosis of histoplasmosis has been presented. The series was analyzed from the standpoints of: symptomatology, physical findings, skin tests, serologic reactions, and the roentgenographic patterns, as well as the surgical procedures and findings on the 66 resected cases.

Sumario

Histoplasmosis: Presentación de Cien Enfermos Hospitalizados Consecutivamente

Preséntase un análisis de los hallazgos en 100 enfermos hospitalizados consecutivamente cuyo diagnóstico definitivo fué histoplasmosis. Se discuten los procedimientos y hallazgos

quirúrgicos para los 66 sujectos cuyas lesiones se excindieron. Descríbense la semiología, los hallazgos físicos, las cutirreacciones, la serología y los patrones radiográficos para todo el grupo.

Resume

Histoplasmose: Présentation de 100 malades hospitalisés consécutivement

Présentation d'une analyse de données obtenues chez 100 malades hospitalisés consécutivement. Le diagnostic définitif d'histoplasmose avait été porté. Les procédés chirurgicaux et les signes observés sont commentés à l'occasion de 66 cas d'exérèse. La symptomatologie, les signes physiques, les tests cutanés, la sérologie et le caractère des images radiologiques sont décrits pour toute cette série de malades.

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THE FATE OF THE PATIENT WITH PERSISTENT CAVITATION AND NONINFECTIOUS SPUTUM ("OPEN-NEGATIVE") AFTER DISCHARGE FROM THE HOSPITAL[1,2]

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INTRODUCTION

In recent years a considerable amount of attention has been directed not only to the pathologic phenomena of so-called "open healing" (1–15) of tuberculous cavities but also to the patient who has acquired a noninfectious status despite persistent cavitation under drug therapy (16–18). It is well recognized that there is an increasing number of such patients who will not be surgical candidates. There is also a group of patients with cavitary disease and sputum negative for tubercle bacilli who are surgical candidates but who will not accept proffered surgical therapy.

The aim of this paper is to present the preliminary experience of the present writers with such a group of patients who have been discharged to continue on drug therapy at home.

Background Information on Group of Patients

Between July 1, 1953, and December 31, 1956, 159 patients with cavitary disease, sputum negative for tubercle bacilli, and pulmonary lesions stable on roentgenography were discharged from Battey State Hospital and continued on drug therapy. Ninety-five of these patients were Negro and 64 were white (table 1).

The white male group ranged in age from 28 to 72 years, the average age being slightly more than 52 years. The white female group averaged approximately 39 years, with a divergence between 21 and 69 years. The Negro male group averaged slightly more than 45 years, the age extremes being 16 and 80 years. The Negro female group averaged 42 years of age with the extremes of 16 and 73 years (table 2).

The average period of hospitalization for each patient approximated two years, and most patients received chemotherapy for the same period. In the white female group there were 2 patients with long periods of hospitalization and shorter periods of drug therapy. One patient had been hospitalized for 75 months and had received chemotherapy for the last 49 months of hospitalization; the other patient had been hospitalized for 91 months and had received drug therapy for the last 39 months of hospitalization. The data for these 2 patients thus account for an approximate difference in this group of six months between the hospital stay average in months and chemotherapy in months.

It is believed important to know something of the type of drug therapy that was given to a group of patients such as these while they were in the hospital. An analysis of their drug therapy shows that one patient received only isoniazid, 68 received isoniazid-PAS, 7 received streptomycin-PAS, one received isoniazid-streptomycin, and 82 patients re-

[1] From Battey State Hospital, Rome, Georgia.

[2] Presented, in part, before the Medical Session, *as part of Section 1A*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

TABLE 1

COLOR AND SEX DISTRIBUTION OF PATIENTS WITH "OPEN-NEGATIVE" SYNDROME TREATED WITHOUT SURGERY AND DISCHARGED FROM JULY 1, 1953, TO DECEMBER 31, 1956

	Males	Females	Total
White	53	11	64
Negro	48	47	95
Total	101	58	159

TABLE 2

AVERAGES OF AGE, DURATION OF HOSPITALIZATION, CHEMOTHERAPY, AND BACTERIOLOGIC STATUS OF SPUTUM BY SEX AND COLOR

	Ages	Hospital Stay	Chemotherapy	Sputum Negative for *M. Tuberculosis* Before Discharge
	years	*months*	*months*	*months*
White males	52.18	22.67	21.94	13.41
White females	39.00	29.18	23.00	13.63
Negro males	45.18	26.86	23.29	18.12
Negro females	42.08	23.70	23.04	15.31

ceived streptomycin, isoniazid, and PAS. In other words, all but 7 of these patients received isoniazid-containing regimens for a considerable length of time.

In table 2 may be seen the length of time these patients had been in a noninfectious status prior to their discharge from the hospital. As a group, the patients had attained a noninfectious status for a year to a year and a half prior to discharge from the hospital. The Negro patients, as a whole, had had sputum negative for tubercle bacilli for an average period of 16.6 months, and the corresponding value for the whites was 15.5 months.

When the study was started it was planned to follow only patients with "open-negative" cavitary disease who had been discharged from the hospital with medical sanction. On close analysis, however, it was discovered that there were 9 patients in this group who had unsanctioned discharges, and they have been allowed to remain in the study.

The bacteriologic studies made in this group of patients while they were hospitalized are presented in table 3. Each white patient had an average of 15 consecutive sputum cultures and more than one gastric culture negative for tubercle bacilli. Prior to discharge, the individual Negro patients had an average of 17 consecutive sputum cultures and slightly less than one gastric culture, all negative for tubercle bacilli.

Surgery was recommended for 37 of these patients (table 4), who refused the procedures and were subsequently discharged from the hospital. One hundred and twenty-two of the patients were not considered surgical candidates because of the extent of their disease or because of such factors as age or poor vital functions.

The chest roentgenograms obtained on all of these patients at the time of discharge from the hospital were carefully reviewed. One hundred and twenty-three patients had single cavities, 11 of them had more than one cavity in one lung, and 25 of them had bilateral cavitation (table 5).

The cavitary lesions were measured and the data are shown in table 6. The patients with single cavities had cavities that were approximately 5 cm. in diameter. The patients

who had multiple cavities in one lung had cavities that were approximately 10 cm. in aggregate diameter. The patients who had bilateral cavities had a combined diameter of their cavities of slightly in excess of 9 cm.

The hospital discharge roentgenogram on each patient was also reviewed from the standpoint of fibrocaseous residue. One hundred and sixteen of the patients had considerable fibrocaseous residue in addition to the previously described cavitary lesions, while 43 had no significant residue (table 7).

TABLE 3

CONSECUTIVE CULTURES NEGATIVE FOR M. TUBERCULOSIS PRIOR TO DISCHARGE FROM HOSPITAL

	White		Negro		Total	
	Cultures	Patients	Cultures	Patients	Cultures	Patients
Gastric washings	79	67	51	94	131	161
Sputum	913	67	1,670	94	2,579	161
Average per patient						
Gastric washings	More than 1		Less than 1		1 per patient	
Sputum	15		17		16 per patient	

TABLE 4

STATUS WITH RESPECT TO POSSIBLE SURGERY OF 159 PATIENTS DISCHARGED FROM BATTEY STATE HOSPITAL ON CHEMOTHERAPY

(From July 1, 1953, to December 31, 1956)

	Surgery Refused	Not Surgical Candidates	Total
White males	8	45	53
White females	1	10	11
Negro males	16	32	48
Negro females	12	35	47
Total	37	122	159

TABLE 5

NUMBER AND TYPES OF CAVITIES IN STUDY OF 159 PATIENTS DISCHARGED ON CHEMOTHERAPY FROM BATTEY STATE HOSPITAL

(From July 1, 1953, to December 31, 1956)

	Single	Multiple in One Lung	Bilateral	Total
White males	40	3	10	53
White females	9	0	2	11
Negro males	35	4	9	48
Negro females	39	4	4	47
Total	123	11	25	159

Observations

Follow-Up Study of the "Open-Negative" Group

The period of time that has elapsed, at the follow-up point, since discharge of the patients from the hospital is shown in table 8. The longest period for which any patient in this group has been followed is 48 months. No patient has been

TABLE 6

Average Size of Cavities in 159 Patients Discharged on Chemotherapy from Battey State Hospital

(From July 1, 1953, to December 31, 1956)

	Number	Single	Multiple Ipsilateral Lung	Bilateral
		cm.	cm.	cm.
White males	53	4.07	8.00	7.30
White females	11	4.00	0	10.00
Negro males	48	5.00	10.00	10.33
Negro females	47	5.57	8.66	9.75
Total average		4.82	9.90	9.36

TABLE 7

159 Patients With Cavities Who Were Given Medical Discharges

(From July 1, 1953, to December 31, 1956)

	Far Advanced	Moderately Advanced	Considerable Residue	No Significant Residue	Total
White males	47	6	42	11	53
White females	9	2	8	3	11
Negro males	44	4	34	14	48
Negro females	44	3	32	15	47
Total	144	15	116	43	159

TABLE 8

Follow-Up Observations Ending March 31, 1957

Duration of Time Since Discharge	White Male	White Female	Negro Male	Negro Female	Total
months					
48		1			1
39–45			3	4	7
33–39	10	1	5	7	23
27–33	9	1	7	4	21
21–27	8	2	5	3	18
15–21	8	2	13	12	35
9–15	11	2	10	5	28
3–9	7	3	5	11	26
Total	53	12	48	46	159

followed for less than 3 months. Only 26 patients have been out of the hospital for less than one year. Twenty-eight of the patients have been followed for approximately one year; 35, for approximately 18 months; 18, for approximately 2 years; 21, for a period of approximately 30 months; 23, for a period of approximately 3 years; 7, for a period of approximately 42 months; and one, for a period of 4 years.

During the period of follow-up observation, 17 of the 159 patients (11 per cent) have been readmitted for re-evaluation and study (table 9). In the white male group of 53 patients, 5 were readmitted for evaluation, and the tuberculosis in 2 of the 5 had reactivated (table 10). One of these patients, 68 years old with bilateral cavitation and extensive fibrocaseous residual disease, was at home for 22 months when tubercle bacilli were found in the sputum, thus leading to readmission to the hospital. He was discharged from the hospital with tubercle bacilli still present in the sputum. The other patient had been at home for 24 months when he developed an upper respiratory infection, tubercle bacilli reappearing in the sputum. He had previously been offered surgery because of a large cavity, and at the time of the present analysis this patient was still hospitalized under therapy.

There were only 12 white females in this group and none of these had been readmitted for re-evaluation.

Of the 48 Negro males, 7 had been readmitted for re-evaluation, and 4 of these were found to have reactivated disease (table 11).

Of this group of Negro male patients (table 11), one, a 74-year-old male, had been out of the hospital for only 6 months. Another, a 65-year-old Negro male, had been out of the hospital for 14 months. Another had been out of the hospital for 15 months and another patient, for 18 months. In 4 of the 7 patients readmitted for evaluation, the disease had reactivated and in 3 of the 4 there were clinical, bacteriologic, and roentgenographic evidences of the reactivation. Tubercle bacilli disappeared from the sputum of one patient without change in the clinical or roentgenographic findings.

Among the 47 Negro females, one of the 5 readmitted for re-evaluation had experienced reactivation of her disease (table 12). This patient had been out of

TABLE 9

FOLLOW-UP OBSERVATIONS ENDING MARCH 31, 1957

Duration of Time Since Discharge	White						Negro					
	Male			Female			Male			Female		
	Total	Number Readmitted	Number Reactivated	Total	Number Readmitted	Number Reactivated	Total	Number Readmitted	Number Reactivated	Total	Number Readmitted	Number Reactivated
months												
21–48	27	3	2	5	0	0	20	1	0	18	1	1
9–21	19	1	0	4	0	0	23	4	3	17	3	0
3–9	7	1	0	3	0	0	5	2	1	11	1	0
Total....	53	5	2	12	0	0	48	7	4	46	5	1

TABLE 10

GROUP READMITTED FOR RE-EVALUATION (A)

White Males	Age	Length of Stay Outside	Reason for Readmission	Bacteriologic Findings After Readmission	Roentgenographic Findings	Clinical Findings	Previous Surgical Candidate	Present Status
	years	*months*						
1. Geo. E. Eassey	33	27	Sputum streaked, with URI*; readmitted for evaluation	Negative	Cavity changed from 6 to 4 cm.	Same	No; 4 cm. cavity in right upper lobe	Discharged
2. John McDuffie	72	6	Readmitted for bacteriologic study as one culture was reported (+) after discharge first time	Negative	No change	Same	No; 5 cm. cavity, left; 3 cm. cavity, left; 3 cm. cavity, right	Discharged
3. O. B. Adair	61	18	Developed bronchial pneumonia; admitted for evaluation; all sputum negative at home	Negative	No change	Same	No; 5 cm. cavity, right	Discharged
4. Lee H. Hardy	68	24	(+) sputum on outside	(+) sputum	No change	Same	No; 3 cm. cavity, right; 2 cm. cavity, left; extensive fibrocaseous disease	Discharged AMA† with MHB‡ with (+) sputum
5. Marvin R. Smith	33	22	URI with (+) sputum	(+) sputum	*Worse*	Same	Yes and refused; 8 cm. cavity	Hospitalized

* URI = upper respiratory infection

† AMA = against medical advice

‡ MHB = maximal hospital benefit

TABLE 11

GROUP READMITTED FOR RE-EVALUATION (B)

Negro Males	Age	Length of Stay Outside	Reason for Readmission	Bacteriologic Findings After Readmission	Roentgenographic Findings	Clinical Findings	Previous Surgical Candidate	Present Status
	years	*months*						
1. Willie Garner	53	22	Welfare insisted he return for advised surgery. All sputum negative outside	Negative altogether 4 years	No change; 3 cm. cavity	Same	Yes; 3 cm. cavity	Discharged
2. Zebbie Powell	45	18	(+) sputum	(+) sputum	*Worse*	*Worse*	No; right cavity, 6 cm.; left cavity, 8 cm.	Hospitalized
3. Mathis Brown	44	15	(+) sputum	(+) sputum	*Worse*	*Worse*	No; cavity, 3-6 cm.; increased infiltration	Hospitalized
4. Cleveland Walker	36	17	(+) d.s.* (with 3 with URI†) negative culture thereafter	Negative	No change	Asymptomatic	No; 3 cm. cavity, right upper lobe; Upper ⅓ with dense fibrosis, left	Hospitalized
5. J. B. Ward	65	14	(+) sputum	(+) sputum	*Worse*	*Worse*	No; fibrocaseous disease left upper lobe, 2 cm. cavity	Hospitalized
6. George Billingslea	74	6	(+) sputum	(+) sputum	No change	Same	No; destroyed right lobe with 6 cm. cavity	Hospitalized
7. Nolan Newton	32	7	Readmitted for advised surgery; 5 cultures and 2 gastric washings negative for *M. tuberculosis* since discharge	Negative	No change	Same	Yes; 6 cm. cavity right apex	Hospitalized

* d.s. = direct smear

† URI = upper respiratory infection

TABLE 12

GROUP READMITTED FOR RE-EVALUATION (C)

Negro Females	Age	Length of Stay Outside	Reason for Readmission	Bacteriologic Findings After Readmission	Roentgenographic Findings	Clinical Findings	Previous Surgical Candidate	Present Status
	years	*months*						
1. Mattie Allen	39	16	Cough and weight loss; re-evaluation after pregnancy	12 cultures and 3 gastric washings negative for *M. tuberculosis*	No change	Same	No; 5 cm. upper-lobe; fibrosis upper ⅓ right	Discharged
2. Laura Peterson	24	10	Evaluation following pregnancy	Negative	No change	Same	No; 6 cm. cavity, right apex	Discharged
3. Jessie Mae Williams	34	22	Returned for advised surgery	(+) sputum	No change	Same	Yes; 8 cm. cavity, left upper lobe	Hospitalized
4. Irene Hicks	56	11	Loss of weight; all sputum negative for *M. tuberculosis* outside (24 pound weight loss)	Negative	No change	Weight loss	Yes; 2 cm. cavity, left upper lobe	Discharged
5. Katherine James	37	4	Re-evaluation after pregnancy; all sputum negative for *M. tuberculosis* outside	Negative	No change	Same	Yes; 5 cm. cavity, left upper lobe	Hospitalized

the hospital for 22 months. When tubercle bacilli were again found in the sputum, the patient returned for surgery which had been advised during the first period of hospitalization because of an 8 cm. cavity in the left upper lobe.

From this group of patients readmitted to the hospital for re-evaluation, it was found that 7 (4.4 per cent of the total) had experienced reactivations of their tuberculosis while on continuous therapy with isoniazid-PAS outside the hospital.

In addition to the 7 cases of reactivation that were found on studying patients readmitted to Battey State Hospital, there were 4 more patients who were found to have had reactivations of their disease.

One was a 34-year-old Negro male veteran, discharged with a 4 cm. cavity after four months, with sputum negative for tubercle bacilli. This patient has a considerable amount of fibrocaseous residue bilaterally and was not a surgical candidate, either at this hospital or in the Veterans Administration hospital to which he was subsequently admitted. Chest films and planigrams in that institution still show a cavity, and the sputum is intermittently positive for tubercle bacilli. Another of the four reactivations was in a 70-year-old white male with a 2 cm. cavity in the right upper lobe. He had had stable roentgenographic findings, noninfectious sputum for a six-month period, and considerable fibrocaseous disease of the upper half of the right lung. After a year of home treatment, tubercle bacilli are present in the sputum, but there has been no change in the roentgenogram. The third patient was a 60-year-old Negro male who had had noninfectious sputum for the last 19 months of a 43-month period of hospital care. He received isoniazid-PAS for six months at home which was discontinued when he developed gangrene of the leg and had to have it amputated. He believed the drug was the cause of the gangrene and refused to take any more therapy. He now discharges tubercle bacilli in the sputum, and there has been an increase in the disease seen by roentgenography. The fourth patient was a 60-year-old white male with a 4 cm. cavity in the right upper lung field and intermittently infectious sputum. He refuses to come back into the sanatorium; he is considered a public health menace; and the local health officer plans to incarcerate him.

A total of 11 of the 159 patients have had tubercle bacilli reappear in the sputum following a period of noninfectiousness. This is a 7 per cent bacteriologic relapse rate (table 13).

TABLE 13

BACTERIOLOGIC STATUS OF SPUTUM ON FOLLOW-UP OBSERVATION

Sex	White Female	White Male	Negro Female	Negro Male	Total
Positive for *M. tuberculosis*	0	4	1	6	11
Negative for *M. tuberculosis* since discharge from hospital	11	47	43	41	142
Total	11	51	44	47	153

TABLE 14

CAUSES OF DEATH

Sex	White Female	White Male	Negro Female	Negro Male	Total
Extensive burn	0	0	0	1	1
Cerebral hemorrhage	0	0	1	0	1
Cardiac disease	0	2	2	0	4
Total	0	2	3	1	6

There have been 6 deaths in the group (table 14). The death certificates reveal that one patient died of an extensive burn, one died of a cerebral accident, and 4 died of heart disease. The average age of the patients who died was 67 years. No positive bacteriologic findings had been reported for any of this group of patients prior to their death. The total mortality in the group was 3.8 per cent.

Of the 153 living patients, follow-up observations revealed that only 3 were confined to their homes. Seventy-five of them had restricted activity and 69 had unlimited activity. With respect to their ability to work, it was found that 76 were physically unable to work, 32 were working part time, and 44 were working full time. Seventy-one of this group required complete public assistance, 18 required partial public assistance, and 63 were entirely self-supporting.

Roentgenograms were obtained on 110 of the 153 living patients and were compared with the sanatorium discharge film and previous films obtained while the patient was in the hospital. The films of the other 43 patients were interpreted by experienced people who forwarded their interpretations to the present writers. There were only 6 patients who showed roentgenographic worsening. The roentgenograms of 9 patients showed improvement; there were 6 in whom the cavitary disease had vanished as determined by the postero-anterior film.

It was found that the average total duration of chemotherapy in the entire group was 37.7 months. Thirty-eight of the 153 living patients had had their drug therapy discontinued and, to date, there have been no reactivations in that particular group.

The follow-up study on the group that had been offered surgery revealed the following information on them as a specific group. Twenty were on restricted activity and 16 were unlimited in their activity. Twenty-two of them were unable to work, 7 were working part time, and 7 full time. Nineteen of these people were on complete public assistance; 7, on part-time public assistance; and 10, self-supporting. Three of these 36 patients had sputum consistently positive for tubercle bacilli since leaving the hospital, and in 2 there has been roentgenographic worsening. All were receiving chemotherapy at the time of the follow-up observations. There were no deaths and no reported bouts of suppuration or hemorrhage.

Discussion

Bell and associates (19) reported a relapse rate of 43 per cent in a group of 118 patients with the "open-negative" syndrome after 6 to 8 months of treatment with antituberculous drugs. It is not believed that three consecutive monthly negative-sputum examinations qualify a patient as one who has acquired true reversal of infectiousness.

In the present group of 159 patients, the relapse rate has been 8.8 per cent up to the present time. There were 11 patients (7 per cent) with bacteriologic relapse. In addition, there were 3 more patients with reactivation by roentgenographic or clinical worsening without change in bacteriologic status. With respect to reactivation, there was a difference between the sexes, one reactivation occurring among 58 discharged females and 13 reactivations occurring among 101 discharged males. This group of patients had been in a noninfectious status for a mean average of nearly 16 months prior to the time they were discharged from the hospital and, in addition to that, have a period of follow-up ranging from 3 to 48 months. As stated previously, only 26 of these patients have follow-up observations of less than one year.

It should be particularly emphasized that 152 of the patients in the group had received isoniazid while in the hospital. There were only 3 patients in the group who had been advised to take streptomycin-PAS as post-sanatorium drug regimen, while 153 had isoniazid-PAS and 3 had isoniazid-streptomycin.

The death rate of 3.8 per cent in this group occurred in people who had not had bacteriologic or roentgenographic relapse. They were not treatment failures. It was not possible to determine whether the people who were listed as having died from heart disease had died from cor pulmonale or from some other heart disease such as coronary arterial thrombosis.

The group of patients who had refused surgery is quite small. Three of these (8.33 per cent) have had reactivations of their disease during the period of observation.

There are many unanswered questions. How long should a patient with the "open-negative" syndrome be continued on drug therapy? It is believed that this must be highly individualized, but a considerable number of these people should be continued on drug therapy indefinitely. On the other hand, among the group of 38 patients whose drug therapy has been stopped, there have been no reactivations to date.

There has been only one patient from this group who has been considered a public health menace. If such people can be adequately cared for medically in a home situation, they are much happier, and it is cheaper to maintain them on drugs outside the hospital than it is to carry them as inpatients. A considerable number of these people, even with cavitary disease, if given the opportunity, with public health clearance as to noninfectiousness, can become self-supporting and again take their place in society.

Summary

A continuing follow-up study has been made of a group of tuberculous patients on home care for the "open-negative" syndrome. On the basis of this experience, the following conclusions have been reached:

It is safe to discharge the patient with the "open-negative" syndrome to his home environment without creating a public health problem.

The mortality rate in this group of patients was 3.8 per cent. The reactivation rate was 8.8 per cent. There were no additional reported bouts of suppuration or hemorrhage.

There has been no discernible difference up to the present time in the reactivation rate between the Negro and white races. The male sex has not fared as well as the female sex, 13 of the 14 reactivations being in males.

Only 2 per cent of the patients were confined to their homes because of their physical disabilities. Forty-five per cent of them had unlimited activity. More than 25 per cent of the patients were found to be working full time. This serves somewhat as a counterbalance to the previous concept of the present writers that these cavities should be removed surgically or collapsed by thoracoplasty prior to the time when the patients could hope to return to their families and earn a living.

Careful long-term follow-up observations on patients in this category are of extreme importance.

Sumario

El Destino del Enfermo "Abierto-Negativo" Dado de Alta

Este estudio continuo de observación subsecuente ha aportado varias cosas a nuestras ideas de la asistencia del enfermo "abierto-negativo":

No hay riesgo en dar de alta a su ambiente doméstico al enfermo clasificado como "abierto-negativo" sin crear así un problema sanitario.

La mortalidad en este grupo de enfermos representó 3.8 por ciento. El coeficiente de reactivación fué de 8.8 por ciento. No se comunicaron nuevos episodios de supuración o hemorragia.

No ha habido hasta ahora diferencia discernible en el coeficiente de reactivación entre las razas negra y blanca. El sexo masculino no lo ha pasado tan bien como el femenino, correspondiendo al primero 13 de las 14 reactivaciones.

Sólo 2 por ciento de los enfermos se vieron recluídos a sus hogares debido a su incapacidad física. Cuarenta y cinco por ciento gozaron de actividad ilimitada. Más de 25 por ciento de los enfermos trabajaban horas normales. Esto sirve en algo como contrapeso del antiguo concepto de que deben resecarse quirúrgicamente o aplastarse con la toracoplastia las cavernas de estos enfermos antes de poder esperar volver al seno de sus familias y ganarse la vida.

En los enfermos de esta clase las cuidadosas observaciones subsecuentes a largo plazo poseen suma importancia.

Resume

L'avenir du malade "open-négative" après sa sortie de l'hôpital

La poursuite des observations en post-cure a contribué à nous fournir certaines notions sur le traitement du malade abacillifère porteur de cavernes épithélialisées.

Le retour au foyer du malade abacillifère à caverne épithélialisée est une mesure inoffensive et ne crée pas de problème d'hygiène publique.

Dans ce groupe de malades, le taux de mortalité a été de 3,8% et le taux de réactivation de 8,8%. Aucun cas additionnel de suppuration ou d'hémorragie n'a été rapporté.

Il n' y a eu jusqu'à présent aucune différence appréciable entre le taux de réactivation dans la race noire ou la race blanche. Chez l'homme, l'état était moins bon que chez la femme, sur 14 cas de réactivation, 13 se sont produits chez l'homme.

Il n' y a eu que 2% des malades que leur état a contraint à rester au domicile. Dans 45% de cas, les malades avaient une activité normale. On a constaté que 25% des malades assuraient un travail à plein temps. Ces notions paraissent, dans une certaine mesure, contre-balancer notre conception antérieure à l'égard de la nécessité de la résection de ces lésions par la chirurgie, la collapsothérapie ou la thoracoplastie, avant le moment où ces malades pouvaient espérer le retour au foyer et à un travail rémunérateur.

L'observation scrupuleuse et prolongée des malades appartenant à cette catégorie est d'une extrême importance.

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THE DISC-METHOD TUBERCULIN TEST[1]

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Introduction

In 1937, the present writer (1, 2) introduced the multiple-puncture method of BCG vaccination. This method differs from the multiple pressure or puncture method used for smallpox vaccination in three fundamental points. First, it is deeper, the points entering into the upper layers of the cutis rather than of the epidermis as in the smallpox vaccination method. Second, after the original puncture of the skin is made by the point of the needle held parallel to the skin, a further injection of the needle point about 1 to 2 mm. into the skin is made in an upward motion of the needle. Third, the multiplicity of injection is spread over a wide area to effect many vaccination sites instead of the single lesion produced with smallpox vaccination.

Birkhaug (3) modified this method by introducing a spring-actuated apparatus with which the punctures were made simultaneously. A further modification was introduced by the present writer (4, 5), who developed a stainless steel disc from which multiple prongs protruded and by which multiple sites were punctured with one operation. The advantage of the latter method was that it was simple in application, inexpensive, and the sterilization of the stainless steel disc could be readily accomplished.

In 1951, Heaf (6) introduced a multiple-puncture method for tuberculin testing, using a spring-actuated apparatus with six needles. Concentrated Old Tuberculin (OT) with epinephrine or purified protein derivative (PPD) (2 mg. per ml. in glycerin) is spread on the skin with a loop, the instrument is placed on the skin and, with the release of a catch, the needle descends into the skin for 1 to 2 mm., depending upon the set of the instrument. Sterilization is effected by dipping the head of the instrument in alcohol and setting fire to it. The object of the present paper is to introduce the disc method for tuberculin testing.

Methods and Materials

The disc method is similar to the one used for BCG vaccination originally described by the writer. Instead of the 36 prongs spaced on a disc $\frac{7}{8}$- by 1-inch as used for BCG, for tuberculin testing, a disc $\frac{2}{8}$- by $\frac{3}{8}$-inch with only 4 prongs is used. A magnet is used as a holder as for the disc method of BCG vaccination. The results on 948 infants, children, and young adults tested with the disc method are compared with the intradermal Mantoux method using various dilutions of tuberculin (1:5,000; 1:1,000; and 1:100). In all, some 2,184 tests were performed.

As a result of this study, it was found that the disc-method tuberculin test has the following advantages: It is relatively painless; it is a one-test-sensitive procedure; it is

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easy to perform; the interpretation of the readings is simple; no dilutions of tuberculin are necessary; the use of a needle and syringe is eliminated since the tuberculin may be put in capillary tubes; and it is inexpensive.

Equipment needed

Disc: A thin stainless steel plate $\frac{3}{8}$- by $\frac{3}{8}$-inch from which four perpendicular prongs protrude approximately $\frac{3}{16}$-inch apart. The length of the prong is 93/1,000 of an inch. One margin of the disc is somewhat wider than all others and is used for spreading the tuberculin (figure 1).

Holder: The holder is composed of a magnetized piece of metal $\frac{1}{4}$-inch square and $1\frac{1}{2}$-inches long (figure 1).

Tuberculin: Concentrated Old Tuberculin (OT) is used. This may be put in small capillary tubes for use in private practice. A saw and small rubber bulb are used to open the capillary tube and express the tuberculin. When large-scale testing is to be done, the ordinary 1 ml. tuberculin syringe and 26-gauge needle is used to remove the tuberculin from the vial.

Method of injection

The outer aspect of the right arm over the deltoid region is used as the site of inoculation. The area is cleansed with acetone and allowed to dry A small drop of concentrated OT is dropped on the arm either from a 26-gauge needle or from the capillary tube containing the tuberculin. The disc is picked up with the magnet so that the wide margin protrudes slightly beyond the magnet. The arm underneath the deltoid area is grasped with the left hand, and the skin is made taut. The control test is made about 1 inch below the site of tuberculin. The disc is applied to the skin and enough downward pressure is exerted so that the points are buried in the skin. The magnet and the disc are then rocked slightly forward, backward, and sideways, the pressure being maintained on the magnet. The pressure underneath the arm is released while the pressure is still maintained on the magnet. After the pressure underneath the arm is released, the magnet and the disc are withdrawn from the skin (this procedure minimizes capillary bleeding). A properly executed injection is one in which the margins of the disc are imprinted on the skin.

With the same disc and magnet, the actual test is performed as follows: The disc is held at about a 30-degree angle to the skin so that the wide margin faces cephalically and down. With this margin the tuberculin is spread by gentle tapping over an area approximately $\frac{1}{4}$ of an inch square. The points of the prongs are gently dipped into the tuberculin so that they are slightly moistened. From then on, the technique is the same as described above.

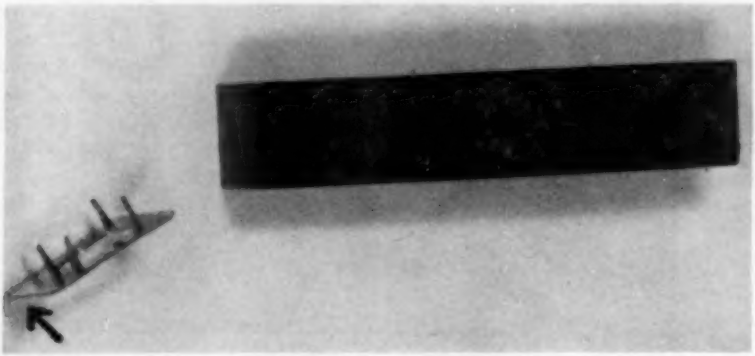

FIG. 1. Disc with four prongs and magnet (× 2.3). Arrow points to wide margin or edge.

After the disc is removed from the skin, it is again held at a 30-degree angle, and the wide margin is used to gently distribute the tuberculin into the four apertures.

In the present study the tuberculin was allowed to dry on the arm, but it has been shown in subsequent studies that gently blotting the excess tuberculin with gauze or cotton did not affect the test. The control injection site was used in all of the 948 subjects in this report, but since there were practically no pseudoreactions from the use of the disc, it can be discontinued from routine practice.

Methods of interpretation

Disc method: The reading of the site is made forty-eight hours after the inoculation. The subject is placed in a position which allows the light from a good source or daylight, if it is adequate, to cast diagonal rays onto the site. The viewer notes the shadows cast by any elevation at the site of inoculation. The induration of this elevated area is confirmed by palpation with the index finger. The extent of the induration is measured in millimeters. Any papule or maculo-papule 2 mm. or more in diameter is considered to be a positive reaction. The extent of the reaction is indicated as follows:

Negative. No lesion or any lesion that is not elevated whether it be hyperemia or slight capillary hemorrhage.

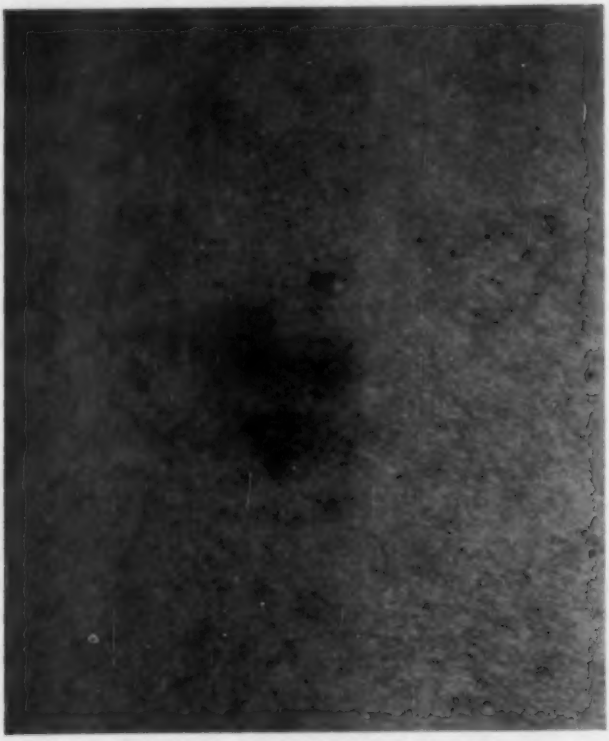

FIG. 2. A disc test showing a 3-plus reaction. Note elevated papule and surrounding erythema with fusion of lesions (× 2).

Doubtful. Elevated lesion measuring up to 1.5 mm. in diameter.

Positive. One-plus (+): Discrete elevated lesions measuring 2 mm. in diameter.

Two-plus (++): Discrete or partially discrete elevated lesions 3 to 4 mm. in diameter.

Three-plus (+++): Two or more papules which have diffused. The measurement of these sites may be made with a millimeter rule (figure 2).

Four-plus (++++): Reserved for necrotic lesions; these were not seen in the present study.

Mantoux method: The reading of the test is done at 48 hours. The size of the induration is measured in two diameters and recorded. Any induration of 6 mm. or more is considered a positive reaction.

The disc test and the first strength by the Mantoux method is applied at the same time.

Method of sterilization of the disc and magnet

Care of the disc: After use, the disc is washed in running tap water and then boiled in distilled water for ten minutes. The disc is placed on four thicknesses of gauze with the prongs downward and is covered with two thicknesses of gauze. The disc and gauze are wrapped in paper, and sterilization is affected by autoclaving at 15 pounds (120°C.) for thirty minutes.

Care of the magnet: The magnet is washed in distilled water, dried, wrapped in gauze, and autoclaved at 15 pounds (120°C.) for thirty minutes. Between tests, the end in use is wiped with sterile gauze soaked in 70 per cent alcohol. A small amount of alcohol is then poured directly over this end. The magnet is kept in a gauze sponge soaked with 70 per cent alcohol until used. Immediately before use, it is wiped dry with a sterile sponge.[2]

Results

The results will be considered in subjects naturally infected or not infected and in those who have been vaccinated with BCG. The two groups are not necessarily the same. Both qualitative and quantitative differences will be considered.

Disc Readings (±) as Related to the Reactions (±) to Various Strengths of OT (Subjects Naturally Infected or Not Infected)

The testing was done in children six to sixteen years of age and in medical students at the end of their freshman year. Of the 316 children tested, there was a 90 per cent correlation between those who reacted to both the disc and Mantoux methods when 1:5,000 dilution of OT was used; an 80 per cent correlation between the disc method and those reacting up to 1:1,000 OT; and a 57 per cent correlation between the disc method and those reacting up to 1:100 OT. For the 186 adults, the correlation ratios were 100 per cent, 93 per cent,

[2] Although by this technique an individual disc is used for each subject, a common magnet is used for many subjects. The writer believes that the possibility of transferring serum hepatitis by the technique used is remote. This belief is based on the facts that the magnet is separated from the skin by the back of the disc and the proximal portion of the injecting points and that pressure is exerted on the magnet to effect hemostasis until it is slipped off the disc. Between injections, the magnetic edge is first wiped thoroughly with 70 per cent alcohol-soaked gauze, washed in clean alcohol, then soaked in an alcohol sponge, and finally wiped with dry gauze immediately before use. In more than 100,000 subjects, when this technique or a modification thereof has been used, no one has reported the occurrence of hepatitis.

TABLE 1A

DISC READINGS (±) AS RELATED TO THE REACTIONS (±) TO VARIOUS STRENGTHS OF OLD TUBERCULIN (SUBJECTS NATURALLY INFECTED OR NOT INFECTED)

	Total Tested	Number of Reactors to 1:5,000*	Number of Reactors to Disc	Per Cent of Reactors to Disc	Number of Reactors to 1:1,000	Number of Reactors to Disc	Per Cent of Reactors to Disc	Number of Reactors up to 1:100†	Number of Reactors to Disc	Per Cent of Reactors to Disc	Number of Nonreactors to OT	Number of Reactors to Disc	Per Cent of Reactors to Disc
Children...	316	91	82	90	116	93	80	200	113	57	116	2	2
Adults.....	186	28	28	100	67	62	93	122	80	66	64	11	17
Total....	502	119	110	92	183	155	85	322	193	60	180	13	7

Correlation disc and Mantoux methods—88.7 per cent.

False positives to disc method—3.5 per cent.

(±) = Positive or negative reactors.

* Reactors to 1:5,000 OT were not tested further. Nonreactors to 1:5,000 were tested with 1:1,000 and 1:100 OT at the same time.

† Includes all reactors including reactors to 1:100 OT.

and 66 per cent for the three strengths of tuberculin, respectively. For the combined groups the discrepancies among those who reacted strongly (to 1:5,000) and among the nonreactors to the Mantoux test were small (8 and 7 per cent, respectively); 15 per cent of the reactors to 1:1,000 OT (Mantoux) were nonreactors by the disc test. Excluding the results of the Mantoux reactions up to 1:100 (since the sensitivity to the disc method is somewhat less than the 1:100 OT dilution), there was an over-all correlation of 88.7 per cent between the two methods. For this group of 363 subjects, the disc test gave 3.5 per cent false positives (table 1*A*).

Disc Reading (±) as Related to the Reactions (±) to Various Strengths of OT (BCG-vaccinated Subjects)

In this group, newborn subjects who were vaccinated two to three days after birth were included as well as children and adults. It includes those who were vaccinated two to three years previously as well as some who had been given 9, 18, and 27 punctures instead of the usual 36. Also included were adults who had been vaccinated by the intradermal method. The children were within the age group of six to sixteen years, but they were not necessarily the same ones included in table 1*A*. The young adults were freshmen medical students. The correlation for the individual groups was similar to those naturally infected or not. Taken as a whole, of the 446 persons tested, there was a 98 per cent correlation between whose who reacted to the disc and Mantoux methods when 1:5,000 OT was used; a 91 per cent correlation between the two methods when up to 1:1,000 OT was used; and a 79 per cent correlation between the two methods when up to 1:100 OT was used. Nonreactors to all strengths of OT by the Mantoux method were too few to make a comparison with those reacting to the disc method

TABLE 1*B*

DISC READINGS (±) AS RELATED TO THE REACTIONS (±) TO VARIOUS STRENGTHS OF OLD TUBERCULIN (BCG-VACCINATED SUBJECTS)

	Total Tested	Number of Reactors to 1:5,000*	Number of Reactors to Disc.	Per Cent of Reactors to Disc	Number of Reactors to 1:1,000	Number of Reactors to Disc	Per Cent of Reactors to Disc	Number of Reactors up to 1:100†	Number of Reactors to Disc	Per Cent of Reactors to Disc	Number of Non-reactors to OT	Number of Reactors to Disc
Newborns.....	27	—	—	—	25	23	92	27	24	89	—	—
Children.....	269	120	116	97	187	170	91	246	195	79	23	2
Adults.....	150	55	55	100	98	90	92	141	110	78	9	3
Total	446	175	171	98	310	283	91	414	329	79	32	5

Correlation of the disc and Mantoux methods—90.6 per cent.

False positives to the disc method—1.5 per cent.

(±) = Positive or negative reactors.

* Reactors to 1:5,000 OT were not tested further. Nonreactors to 1:5,000 were tested with 1:1,000 and 1:100 OT at the same time.

† Includes all reactors including reactors to 1:100 OT.

meaningful (table 1*B*). The discrepancy between the 1:5,000 group and the disc method was very small (2 per cent); whereas in the up to 1:1,000 group it was also small (9 per cent). Considering the reactors up to 1:1,000 OT and the nonreactors to all dilutions of tuberculin (342 tests), there was a correlation of 90.6 per cent between the disc and the Mantoux methods. There were 1.5 per cent false positives, i.e. 5 cases in 342 tests.

The results of the disc and Mantoux tests, both in the naturally infected and BCG-vaccinated subjects, were more or less parallel. Therefore, the disc test is applicable to those naturally infected as well as those vaccinated with BCG. The sensitivity of the test was somewhere between 1:1,000 (10 TU) and 1:100 (100 TU) OT as determined by the Mantoux method.

The Size of the Disc Readings as Compared to the Reactions (±) to Various Strengths of Old Tuberculin by the Mantoux Method (Subjects Naturally Infected or Not Infected)

In table 2 a comparison is presented between the size of the disc reaction and the reaction to various strengths of OT by the intradermal method. From this table it will be noted that, in children who reacted to 1:5,000 OT, 75 per cent had fused lesions (3-plus), whereas only 8 per cent of the reactors to 1:1,000 OT and 2 per cent of those who reacted to 1:100 OT had fused lesions. In the adults, the percentages were 61, 23, and 4, respectively, for the various strengths of OT. For both groups as a whole, the percentages were 71, 17, and 3, respectively. None of the nonreactors had fused lesions. Of those reacting to 1:5,000 OT, only 8 per cent of children and 11 per cent of adults reacted 1-plus to the disc test. Of 180 children and young adults who were nonreactors to all strengths of OT,

TABLE 2

SIZE OF DISC READINGS COMPARED TO THE REACTIONS (±) TO VARIOUS STRENGTHS OF OT BY MANTOUX METHOD (SUBJECTS NATURALLY INFECTED OR NOT INFECTED)

Disc Reading	Children		Adults		Total	
	Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Reactors to 1:5,000 OT						
Fused (+++)	68	75	17	61	85	71
3–4 mm. (++)	7	7	8	28	15	13
2 mm. (+)	7	8	3	11	10	8
1 mm. (±)	1	1			1	1
Nonreactors (−)	8	9			8	7
Total	91	100	28	100	119	100
Reactors to 1:1,000 OT						
Fused	2	8	9	23	11	17
3–4 mm	3	12	8	20	11	17
2 mm	6	24	17	44	23	36
1 mm	9	36	1	3	10	16
Nonreactors	5	20	4	10	9	14
Total	25	100	39	100	64	100
Reactors to 1:100 OT						
Fused	2	2	2	4	4	3
3–4 mm	4	5	3	5	7	5
2 mm	14	17	13	24	27	19
1 mm	29	34	12	22	41	30
Nonreactors	35	42	25	45	60	43
Total	84	100	55	100	139	100
Nonreactors						
Fused						
3–4 mm	1	1			1	1
2 mm	1	1	11	17	12	7
1 mm	27	23	17	27	44	24
Nonreactors	87	75	36	56	123	68
Total	116	100	64	100	180	100

one had a 2-plus reaction, 12 had 1-plus reactions (7 per cent), and 167 (92.7 per cent) had either doubtful reactions or no reaction.

There seems to be a more or less direct relationship between the size of the disc readings and the reaction to various doses of OT used in the Mantoux method.

Size of the Disc Reading as Compared to Reactions (±) to Various Strengths of Old Tuberculin by the Mantoux Method (BCG-vaccinated Subjects)

In this group, newborns were included but, because of the small number (27 subjects) and since the findings were similar, they were grouped with children. The results in children and adults paralleled those obtained in the groups naturally infected or not infected. For example, of the total group of 175 reactors to 1:5,000 of OT, 66 per cent showed fused reactions, 26 per cent showed a 2-plus reaction (3 to 4 mm.), and 6 per cent had a one-plus reaction. Of those reactors to 1:1,000 OT, 24 per cent had fused reactions and 38 per cent had one-plus reactions. Of the 102 reactors to 1:100 OT, only 6 per cent had fused reactions. Of the 32 nonreactors to all strengths of OT by the Mantoux method, 20 were negative by the disc method, 7 had doubtful reactions, and 5 had positive reactions. In figure 3, the results of all groups are graphically represented. It will be noted that there is a parallel between the size of the disc test and the reaction (±) to various doses of OT by the Mantoux method.

Size of the Disc Readings as Related to the Size of the Mantoux Reaction to Various Strengths of Old Tuberculin (Subjects Naturally Infected or Not Infected)

In figure 4, a scatter diagram is presented for children and adults of various categories with respect to tuberculin sensitivity, that is, those who reacted only to 1:100; the reactors to 1:1,000, but nonreactors to 1:5,000; and the reactors to 1:5,000. As a rule, the smaller the disc reaction, the smaller the diameter of the Mantoux reaction. This is especially true for the reactors to 1:5,000 OT. In this group of the 10 who reacted with one-plus (+) reaction to the disc test, 5 had Mantoux reactions of 9 to 10 mm. or less, and 5 had reactions of more than 11 mm. Of the 15 reacting 2-plus to the disc test (++), 8 had Mantoux readings of 9 to 10 mm. or less and 7 had reactions of 11 mm. or more. Of the 85 who

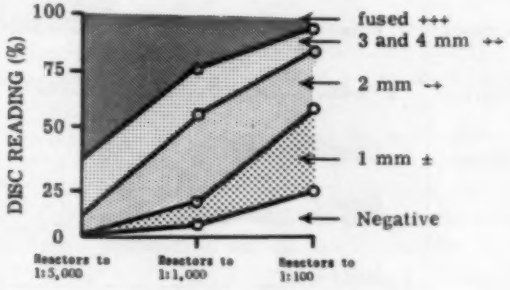

FIG. 3. Size of the disc reading as compared to reactions (±) to various strengths of OT by the Mantoux method (BCG-vaccinated subjects). ± indicates positive or negative reaction.

had 3-plus reactions (fused), 19 had Mantoux readings of 9 to 10 mm. or less, whereas 66 had lesions of more than 11 mm., 27 of whom had lesions of 20 mm. or more.

Generally speaking, there is a more or less direct correlation between the size of the Mantoux reaction and the size of the disc reaction. This was best demonstrated in the 1:5,000 OT reactions.

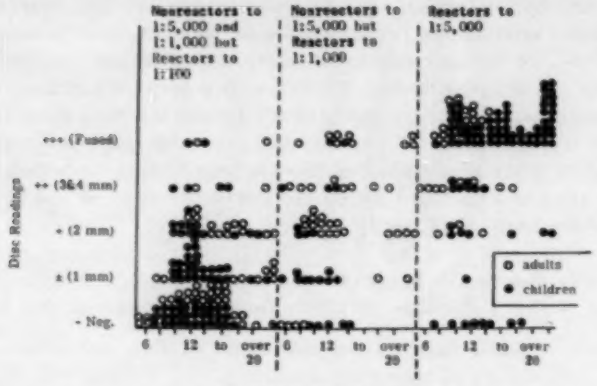

FIG. 4. Size of the disc reading as related to the size of the Mantoux reaction of various strengths of OT (subjects naturally infected or not infected).

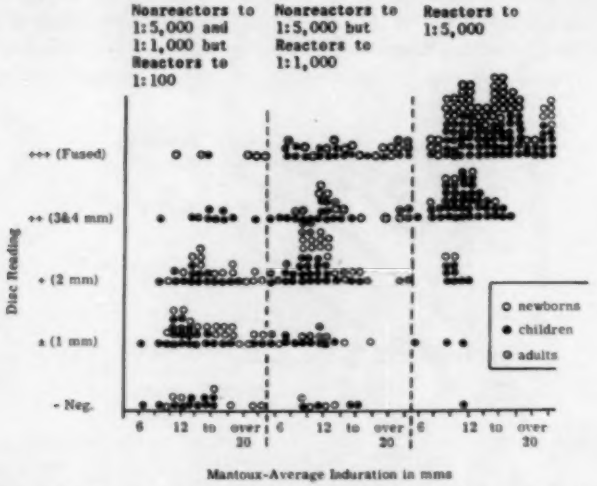

FIG. 5. Size of the disc reaction as related to the size of the Mantoux reaction to various strengths of OT (BCG-vaccinated subjects).

Size of the Disc Reaction as Related to the Size of the Mantoux Reaction to Various Strengths of Old Tuberculin (BCG-vaccinated Subjects)

Figure 5 is a scatter diagram of the size of the disc reaction as compared with the size of the Mantoux reaction to various strengths of tuberculin in subjects vaccinated with BCG. A more or less direct correlation was noted for all categories of tuberculin sensitivity and, as would be expected, was best seen in the reactors to 1:5,000 OT. In this group, of the 10 reacting one plus (+) by the disc method, 8 had lesions of 9 to 10 mm. or less. Of the 45 with the 2-plus reaction (++), 18 had Mantoux readings of 9 to 10 mm. or less and 27 had readings of 11 mm. or more. Of those reacting 3-plus to the disc method, 22 of a total of 116 had reactions of 9 to 10 mm. or less, whereas 94 had reactions of 11 mm. or more. Of these 94, 18 had average diameters of 20 mm. or more.

The average diameter of the tuberculin reactions found by the Mantoux test for the various dilutions of tuberculin more or less correlated with the size of the reaction following the disc method. This is true for both those naturally infected and those vaccinated with BCG. These findings were noted in children, adults, and in BCG-vaccinated newborns.

Discussion

The disc tuberculin test is an accurate one-test procedure which may be interpreted both qualitatively and quantitatively. The degree of sensitivity lies somewhere between 1:1,000 (10 TU) and 1:100 (100 TU) Old Tuberculin, as determined by the Mantoux method. When compared to 10 TU by the Mantoux method, the correlation is approximately 90 per cent or better. The percentage of false positives lies from 1.5 to 3.5 per cent. No severe reactions were noted in any of the tests performed even though many subjects responded strongly to 1:5,000 OT. The advantages of this test are as follows:

It is relatively painless; this is especially advantageous when testing children. The fact that a needle and syringe is not shown to the child is also of import. It is easy to perform and easy to interpret. Since the lengths of the prongs are fixed, it is not possible to go too deep, as one sometimes does when using the intradermal method. Because of the superficial injection, the reading is simplified, since a definite elevation is more likely to be seen in a reactor. By the Mantoux method, for example, if the injection is too deep, an elevation may not be noted and palpation may be necessary. No dilutions of tuberculin are necessary. This simplifies the procedure, especially for the private physician, and is also economical as far as the tuberculin is concerned because concentrated tuberculin may be kept for a much longer period than diluted tuberculin. The use of needle and syringe may be eliminated; as tuberculin may be put up in capillary tubes, it is not necessary to have a needle and syringe to do the test. When large-scale testing is to be done, a small dropper or applicator may be used to place tuberculin on the arm. This not only simplifies the operation as far as the doctor is concerned but, as stated above, also makes the test more acceptable to the child. It is inexpensive. As the disc may be used over and over again and is less likely

to become blunt than are the injection needles, both the initial cost and the subsequent upkeep are reduced. Furthermore, as it is not necessary to dilute the tuberculin, the use of bottles, pipettes, or syringes and diluent is eliminated. This test for practical purposes is equally as sensitive for the newborn, children, and adults; it is one simple test that may be applied to all age groups.

Summary

The disc method for tuberculin testing is a simple, accurate, and virtually painless procedure which has the added advantage of being easy to perform and easy to read. When compared with the Mantoux reaction, it has a sensitivity between 100 and 10 TU. The test is applicable and equally sensitive for the newborn, children, and adults. The test may be interpreted both qualitatively and quantitatively.

Sumario

El Método del Disco para la Prueba de la Tuberculina

La técnica del disco para la comprobación con tuberculina es un procedimiento sencillo, exacto y prácticamente indoloro, que posee además la ventaja de ser fácil ejecutarlo y fácil leerlo. Comparada con la reaccion de Mantoux, tiene una sensibilidad entre 100 y 10 UT. La prueba es aplicable a recién nacidos, niños y adultos, siendo igualmente sensible en todos ellos y pudiendo interpretarse cualitativa y cuantitativamente.

Resume

Le test à la tuberculine par la méthode du disque

La méthode du disque pour le test à la tuberculine est un procédé simple, précis et pratiquement indolore qui offre l'avantage de la facilité d'exécution et de lecture. Par comparaison avec la réaction de Mantoux, la gamme de sensibilité s'étend de 100 à 10 UT. Ce test est praticable et présente une même sensibilité chez le nouveau-né, l'enfant et l'adulte. Le test peut être interprété qualitativement et quantitativement.

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ASSAY OF THE FATE OF MYCOBACTERIA IN CELL AND TISSUES CULTURES[1]

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Introduction

A quantitative description of the number and fate of mycobacteria in cell or tissue cultures is complicated by a number of factors: the focal character of mycobacterial infection, the wide spectrum of bacterial numbers per cell following phagocytosis and the fact that some cells contain innumerable bacilli, the changing ratios and numbers of bacilli per cell as influenced solely by growth or decline of cell populations, the loss of infected cells from populations on glass surfaces, and the variable lengths of the mycobacterial units during their growth or destruction within the cell population.

Although single factors have been recognized or controlled by different investigators, major difficulties in this laboratory and elsewhere have arisen from continual attempts to ascertain bacillary numbers in preparations suitable for observation of cytologic relationships between cells and bacilli; also from failures to cope with the preferential loss of cells which carry, and may be generating, an important portion of the bacillary populations.

With cultivatable species, the seeding of droplet plates (1) with intact cells and with mycobacteria liberated from washed cells should facilitate enumeration of infected cells and intracellular bacilli. The use of such methods by Pomales-Lebrón and Stinebring (2) to determine the fate of brucellae in monocytes from normal and immune guinea pigs has afforded an elegant confirmation of Suter's (3) microscopic observations with tubercle bacilli. In streptomycin-controlled systems (4), however, organisms which are free or in degenerating cells could not be assayed. Thus, as in the case of the noncultivated mycobacteria, one must rely upon microscopic methods for measuring total changes in bacillary populations.

It seems timely, therefore, to summarize and illustrate a few of the ways in which microscopic determinations may be misleading; to demonstrate that analyses cannot be confined to the numbers of organisms within cells; and to describe a method whereby differences in bacillary counts may be measured and tested for significance without accumulating a series of total counts from each preparation.

Materials and Methods

Macrophage cultures: Human leukocytes were derived by centrifuging defibrinated or heparinized human blood in columns not more than 2.5 inches in depth; by coagulating the interfacial cell layer to a depth of 2 mm.; and by cutting cubes of 1.5 by 1.5 mm. For

[1] From the Leonard Wood Memorial Laboratory, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts.

plasma cultures the rinsed explants were resuspended in 40 per cent of fresh (less than forty-eight hours) human serum and transferred to tubes coated with one drop of chicken plasma (citrate, 0.2 per cent). After clot formation, 0.5 ml. of liquid supernate was added. For explantation into liquid medium, the fragments were resuspended and transferred in 5 per cent chicken plasma (excess discarded); 0.5 ml. of liquid medium was added after about fifteen minutes.

Optimal nutriment proved to be 40 per cent of human serum in a balanced salt solution providing 7 mg. of calcium and 4 mg. of phosphorus per 100 ml., so that the final medium contained 8 mg. of calcium per 100 ml. Optimal maturation of monocytes to macrophages required delay in the first change of medium until the tenth or fourteenth day. Supplementation with bicarbonate and additional medium prevented the pH in the more active cultures from falling below 7.2. During the first renewal, abnormally large or small cultures were discarded. Thereafter, optimal maintenance was provided by feeding each culture with volumes of medium (pH 7.7) proportional to its r^2 value and by renewals whenever the pH had fallen to 7.2.

These cultures were infected with *M. leprae* in three ways:

A. Explants were imbedded in plasma containing a known concentration of *M. leprae*. The bacilli were first encountered by the rapidly migrating polymorphonuclear leukocytes and, after four to six hours, by the slower moving monocytes. During maturation, these cells acquired bacilli from the plasma and from the dying polymorphonuclear leukocytes. The peripheral zone of macrophages continued to acquire bacilli for several weeks.

B. Known numbers or concentrations of *M. leprae* were added to defibrinated or heparinized blood. The mixture was rotated several hours to permit phagocytosis prior to explantation of the buffy coat into plasma or into liquid medium. Approximately 80 to 90 per cent of the bacilli were carried into the culture zone by migrating polymorphonuclear leukocytes and subsequently acquired by the maturing macrophages.

C. The first renewal medium, containing the desired concentration of bacilli, was added in volumes proportional to the r^2 area of macrophage cultures which had matured in liquid medium for two weeks. This was the only method which produced a consistent and reproducible incidence of infected cells.

For microscopic determination of the number of infected cells or of bacilli per cell in plasma-imbedded cultures, it was necessary to affix intact colonies to glass slides and to count along the radii of the cell zones. Comparable information was obtained from cultures in liquid medium by examining spreads of cells collected from pooled supernates following the renewal of nutriment. If such cultures were to be terminated, the cells were released from the glass by means of trypsin.

Fibrocytic cultures: Proliferating cultures of normal mouse or rat fibrocytes were maintained in glass vessels by feeding as required with 30 to 40 per cent of human serum or ascitic fluid supplemented by chick or beef embryo extract in accordance with desired growth rates. Cells of Earle's "L" strain or its clone 929 were maintained in similar concentrations of horse serum, or in 2 per cent serum, Eagle's mixture, and Bacto-peptone®, 0.5 per cent (5).

Known numbers or concentrations of *M. lepraemurium* or other mycobacteria were added in known ratio to the cell populations, as in method *C* above. Results were assessed in cultures fixed *in situ* on glass slips; in cells released and randomized in pancreatin and filmed on glass slides by means of a 1 mm. loop; and in pools of bacilli collected from disrupted cells and accumulated culture supernates and filmed on glass by means of a standard 0.7 mm. loop (6).

Fixation and staining: Plasma cultures were extracted in balanced salt solution and transferred to glass slides. As soon as evaporation assured their adherence, they were fixed and dehydrated for ten minutes in 95 per cent alcohol prior to air drying. Intact cells released by trypsin or pancreatin were rinsed to bring the enzyme concentration to 0.01 per cent and were resuspended in 1 per cent of serum before films were prepared. The

pancreatin in pools of liberated bacilli was inactivated by 0.5 per cent phenol and serum added to a concentration of 1 per cent. Special precautions for fixation of these preparations (5) and methods for staining and differentiation (6) have been given elsewhere.

Experimental Results

Focal character of mycobacterial infections: Analyses of bacillary distributions following explantation of tissues from either natural or experimental mycobacterial lesions reveal: the extremely variable numbers of bacilli in different explants; the widely varying ratios of bacilli per cell within any fragment; the apparently different destructive capacities of individual cells or cell clusters, as evidenced by the "healthy" appearance of bacilli in some cells and "poor" bacilli and/or "rosy" cytoplasm within others. The difficulties of evaluating microscopic observations in cultures of biopsied tissues have been summarized elsewhere (7, 8).

Infection of standard proportions of cells in vitro: It is not difficult to obtain fairly consistent proportions of infected cells if known concentrations of microorganisms are added to standard cell populations planted as aliquots of pipetted cell suspensions. The acquisition of bacilli and/or particles by human macrophages, normal fibrocytes, or "L" cells living on glass[2] follows the patterns described for phagocytosis by leukocytes in tumbling devices (9). The effect of increasing the relative numbers of bacilli per cell must be described by two different mathematical relationships.

With increasing concentrations of bacilli, the average numbers of bacilli acquired per cell increase as a linear function of the bacillary dose. The percentage of bacilli captured per unit time is never proportional to the increased concentration of bacilli.

The increase in the percentage of cells acquiring bacilli, on the other hand, is a function of the log of the numbers of bacilli added. Each doubling of the bacillary dose per culture raises the percentage of cells engaging in phagocytosis by about 14 per cent. Since the percentage of cells containing bacilli can be determined rapidly and irrespective of bacillary clumps or fairly dense cell crowding, this is the convenient method of adjusting bacillary dosage for various experimental purposes.

Variables which have been found to prevent the establishment of the intended relationships may be summarized as follows:

1. Although the rotation of appropriate mixtures of mycobacteria and blood results in reproducible bacterial content in polymorphonuclear and mononuclear cells, the ratios of macrophages maturing in individual cultures initiated from standard explants is not uniform. The larger cultures exhibit a lower ratio of bacilli to cells. Counts must be made from representative large, medium, and small cultures (usually

[2] Rates of acquisition are controlled primarily by the degree to which the desired ratios of cells and bacilli are concentrated, i.e., by frequency of collision or contact; also by cell type and physiologic circumstance. Polymorphonuclear and mononuclear cells acquire bacilli most rapidly and at comparable rates; normal mammalian fibrocytes and the "L" cell, at considerably slower rates; effective phagocytosis by strain HeLa from a dermatoid carcinoma must be stimulated by modification of serum background (10).

a total of four or five) to obtain useful average estimates of bacillated cells or bacilli per cell.

2. This variable yield of macrophages is the reason that macrophage populations established from buffy coat explants required bacillary doses in proportion to the r^2 value of each culture. Otherwise, large cultures showed a lower than average incidence of infected cells, while small cultures were overloaded.

3. In any system, if known numbers of cells are present but unevenly distributed, lower percentages of infected cells are found where the cell population is dense, while abnormally high values occur throughout the sparsely populated portions of the cultures.

TABLE 1

THE WIDELY DIFFERENT NUMBERS OF MYCOBACTERIA ENGULFED BY INDIVIDUAL CELLS (STRAIN "L").*

Number of Bacilli per Cell	Number of Cells per Class		Total Bacilli† Acquired per Class			Useful Objective
0	64		0			Oil
1	7		7			
2	1		2			
3	3		9			
5	2		10			
6	3		18			
7	1		7			
9	2	26	18	214		Oil
11	1		11			
12	1		12			
13	1		13			
15	2		30			
23	1		23			
24	1		24			
30	1		30			
32	4	6LP+§	128	176		
48	1		48		466	Low power
70‡	3		210			
80‡	1	4LP+ST§	80	290		
Totals:	100		680			

Average bacilli per cell	6.8
Average bacilli per phagocytic cell	18.9
Percentage of cells with bacilli	36

* Method: *M. lepraemurium* was added to "L" cell cultures for six hours in the presence of serum albumin and globulin with small molecular supplements. Cells were suspended by gentle scraping (if released by trypsin or pancreatin, cells containing more than 30 to 40 bacilli tend to disappear) and filmed on glass slides.

† Column 3 = product of columns 1 and 2.

‡ As accurate as possible.

§ LP+ = low-power positive for bacilli; LP+ST = stuffed cells.

Distribution of the bacillary load within cells: When increasing proportions of cells acquire bacilli because of increased bacterial dosage, the less actively phagocytic members of the population are permitted or forced to ingest microorganisms, while the more active cells become engorged. For this reason, even the newly established, standardized infection does not permit convenient microscopic analysis of numbers of bacilli per cell. Although the numbers of bacilli per cell shown in table 1 concern the "L" cell and are somewhat more irregular than those observed in macrophages, they serve to emphasize several points. Only 214 of the 680 bacilli captured were found in cells containing less than 30 bacilli, the upper limit which can conveniently be enumerated during oil immersion examination. The remaining 466 bacilli occurred within ten cells, in which the approximate bacterial content could be observed at 100 times magnification.

Thus, in any cell type, when approximately 40 to 50 per cent of the cells have captured bacilli, there are some cells in which, for practical work, the bacterial content must be estimated. The errors introduced may outweigh many precise observations in cells containing modest numbers of bacilli.

The effect of growth or decline of cell populations: Because of growth or decline in either the cellular or the bacillary populations, original distributions of bacilli tend to be distorted by the time results are collected. If total cells are not determined at each sampling interval, data restricted to the percentage of cells with bacilli (or the average bacilli per cell) may be merely measurements of a constantly changing cell population.

Early studies by Smith, Willis, and Lewis (11) revealed that dividing cells containing mycobacteria tend to divide their load equally between two daughter cells. In the absence of comparable rates of bacillary growth, the result is a continual dilution of bacilli until they no longer can be found in such cultures. Nondigestible control particles have been used as a means of distinguishing between the dilution and the destruction of *M. leprae* populations in human fibrocytes from leprous lesions (7, 8).

The decline of cell populations, for example in cultures of irradiated cells or of human macrophages, can produce a fallacious impression that mycobacteria are proliferating. The data in figure 1 and its attendant explanation show five curves in which increases in the percentage of macrophages containing bacilli (also of bacilli per cell, not shown) must be attributed primarily to different rates of decline in cell populations. The average proportion of bacillated cells in *B′* (liquid medium) is lower than in *B* (plasma) during the first four weeks for two reasons: the more successful maturation of macrophages in liquid medium and the lower success in recapturing damaged cells or the bacilli they release.

In the absence of counts of total bacilli per culture, three types of control evidence show these apparent increases in numbers of *M. leprae* to be misleading. Similar curves are produced by employing killed bacilli. If carbon particles are added, the incidence of cells containing carbon rises more rapidly than the incidence of cells with bacilli. After periods of four weeks, destruction of *M. leprae* becomes so rapid that the curves assume downward slopes in spite of continued, slow decline in cell numbers.

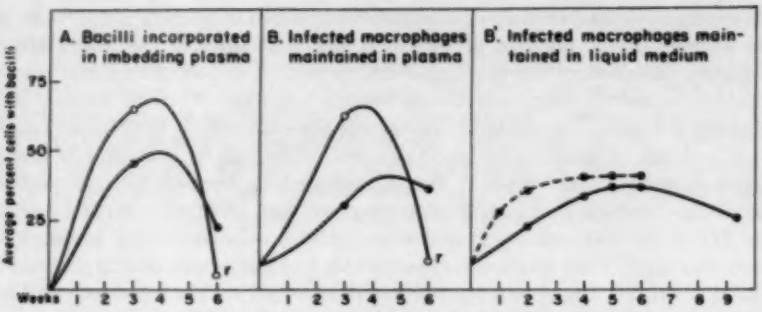
A. Bacilli incorporated in imbedding plasma
B. Infected macrophages maintained in plasma
B'. Infected macrophages maintained in liquid medium
Average percent cells with bacilli
75
50
25
Weeks
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6 7 8 9
(r)

FIG. 1. Relationship between the size of cell populations and the apparent "growth" of *M. leprae* in human macrophages.

Human serum, 40 per cent: ● = calcium, 8 mg. per 100 ml.; P = 4 mg. per 100 ml.; ○ = calcium, 8 mg. per 100 ml.; P = 1.6 mg. per 100 ml.

A and *B*. The lower frequency of infected cells in ● is due to more stable cell populations. Eventual accelerated destruction of bacilli in ○ is denoted by (r) rosy cytoplasm. *A*. Highest frequency of infected cells near the periphery of colonies; greatest concentrations of bacilli acquired by the largest cultures. *B*. Highest frequency of infected cells at the centers of colonies; great concentrations of bacilli in the smallest cultures. *B'*. Solid line = cells on glass; broken line = cells in pooled supernates. The average number of matured cells was larger and the incidence of infected cells in individual cultures was more uniform than in *A* or *B*.

The loss of infected cells from cultures on glass: Within any culture on glass, a certain proportion of the cells have a poor anchorage or float in the medium. During the early weeks of maintaining noninfected cultures of human macrophages, about 16 per cent of the viable cells float, since the sediment from six pooled supernates will establish one new culture equivalent to the originals. In the case of normal mammalian fibrocytes or the "L" cell, the proportion of floating cells is normally very low. When mycobacteria are added to cultures of either cell type, the cells acquiring large numbers of bacilli are damaged. Repeated cycles of infection are maintained because normal or less heavily infected cells continually ingest those which are overloaded. Since the latter are not captured efficiently, they tend to float and to be discarded during renewal of the medium and terminal manipulations.

It may be noted in panel B′ of figure 1 that the percentage of bacillated cells in supernates after two weeks was 12.5 per cent greater than among cells adhering to glass. The floating population, therefore, may have been carrying nearly twice as many bacilli per cell. The data in table 2 demonstrate that, when slightly more than 50 per cent of human macrophages were found infected after cycling *M. leprae* for eleven days, the incidence of bacilli in cells of healthy appearance on glass and in supernates was comparable. Among the damaged cells in the supernate the incidence of bacillated cells was about 19 per cent higher. This indicates that the average concentrations of bacilli in such cells may have been more than twice those in the cells which appeared healthy.

Because of the presumed relative nontoxicity of human and murine leprosy

TABLE 2

ASSOCIATION BETWEEN HIGH CONTENT OF *M. leprae* AND THE RELEASE OF DAMAGED HUMAN MACROPHAGES INTO SUPERNATANT MEDIUM

Culture Number	Incidence of infected cells:			Difference (*b* − *a*)
	On Glass	In pooled supernates		
		(*a*) Healthy Cells	(*b*) Poor Cells	
	per cent	*per cent*	*per cent*	*per cent*
1	46	48	68	20
2	52			
3	48			
4	54	52	70	18
5	39			
6	45	44	62	18

Ten-day cultures of macrophages were infected by method *C* and agitated twice daily. After 11 days at 37°C., supernates recovered after gentle agitation were pooled by pairs and the floating cells collected. The incidence of infected cells was determined among (*a*) cells of the quality which reside on glass and (*b*) those of poorer quality. As a baseline, the cells adhering to the culture vessels were released and the average incidence of infected cells was observed.

bacilli, more extreme examples are of interest. When human macrophages were given sufficient bacilli so that nearly all cells acquired organisms, the cultures within one week exhibited a preponderance of large rounded cells, most of which were released from glass by gentle agitation of the medium. The residual populations were comprised almost entirely of minute cells which were incapable of ingesting bacilli until they in turn had matured to the size of monocytes. If unusual numbers of *M. lepraemurium* were added to cultures of normal rat fibrocytes, the cells no longer resembled fibrocytes, but became enlarged and rounded with pyknotic nuclei displaced to the margins of the cytoplasm. They resembled the cells seen in lepromas of murine leprosy. They floated on the surface of the medium in such numbers as to form a visible film. Samples exhibiting 100 per cent of stuffed cells may be transferred to slides by means of a bacteriologic loop.

It has been the experience of this laboratory that there are two methods which prevent the loss of cells and bacilli during the course of tissue culture experiments. These are: the planting of infected cells on solid surfaces which permit renewal of nutriments without disturbing the cell colonies, and Suter's formvar veil (4) over the surface of coverslips. The latter method has not been found useful for assaying modest changes in bacillary populations. Irrespective of the modifications employed, it has been difficult to get attachment of constant numbers of cells per coverslip. Since the cells often are not distributed uniformly and addition of the veil further disturbs their arrangement, the focal character of the mycobacterial infections was accentuated. Consequently, neither total cells nor bacilli

have been estimated reliably by examining any practical number of sampling sites on coverlipis.

Elongation of mycobacterial units: The increases in cell volume which precede the multiplication of bacteria and tissue cells are expressed in mycobacteria by elongations of bacillary bodies. The significance of this phenomenon during early growth *in vitro* was first recognized in *M. avium* by Brieger and Fell (12), and later in *M. tuberculosis* by Fenner and Leach (13), and in *M. ulcerans* and *M. balnei* by Leach and Fenner (14). These elongations are equally characteristic of mycobacterial growth in tissues and cells. Fenner (15) observed that the elongations of *M. balnei* (during two days) and *M. ulcerans* (two to eight days) in the livers of chick embryos corresponded to the growth rates of the respective species. Mackaness and associates have relied upon elongation of RIRv (16) and the bovine strain "Branch" (17) to precede growth within rabbit monocytes. Shepard (18) has emphasized the remarkable elongations of *M. fortuitum* during the first twenty-four hours of its growth within cells of strain HeLa. Hanks and Backerman (19) described unusually long forms of *M. lepraemurium* during its proliferation in cells of the anterior chambers of the eyes of rats and mice. Hilson and Elek (20) measured the elongations which occurred during the early growth of this organism within rat testes. Fenner's data on *M. ulcerans* and *M. balnei* (15) suggest that shortening or fragmentation of bacilli indicate failures to multiply within cells. Although recognition of these important cytologic alterations in some species may require disruption of cells and preparation of films, they have the merit of being susceptible to analysis without quantitative preparation of samples.

Total mycobacterial counts: In view of the complications outlined in foregoing sections, it appears that the fate of mycobacteria in cell cultures may be assessed most easily by combining the bacilli in accumulated culture supernates with those liberated from the corresponding cells and by making direct plate or microscopic counts. When the microscopic method must be used, tedium and uncertainties can be reduced by preparing conveniently reproducible films and by utilizing the numbers of bacilli seen in each field as an entry for statistical analysis.

TABLE 3

ESTIMATION OF THE NUMBER OF BLOCKS WHICH SHOULD BE COUNTED TO YIELD 140 TO 300 BACILLI PER STRIP

Estimated Bacilli per Block* at Rims	Number of Blocks to Count	Probable Number of Bacilli per Strip
40	1	288
30	1	216
20	2	288
15	2	216
10	3	216
5	4	144

* One block = 4 squares arranged horizontally.

By observing several blocks, the size of the "field" is increased as required.

Each sample in 1 per cent serum is stirred with a standard 0.7 mm. loop (AHT # 8304-G) while the 0.000,22 ml. aliquots are transferred to designated sites on a slide laid on graph paper. Six microspots of each sample are arranged vertically; as many as nine samples can be filmed on one slide. The droplets are allowed to spread to their natural limits while drying. The bacilli are distributed uniformly across a central plain surrounded by a definite rim in which they are 2 to 2.5 times more concentrated. It is the faithful replication of this drying pattern which permits the numbers of bacilli in each field to be compared with the numbers observed in the corresponding sites in other films.

The art of preparing suitable films is acquired and evaluated without enu-

TABLE 4

ANALYSIS OF TWO COMPARISONS OF BACILLARY COUNTS, EMPLOYING CRUDE DATA AS RECORDED

Comparison No. 1						Comparison No. 2					
No. 1 Experimental	A Control	Difference (Number 1 − A)		Rank		No. 1 Experimental	A Control	Difference (Number 1 − A)		Rank	
		+	−	+	−			+	−	+	−
36	22					47	34				
20	13					24	10				
14	6					9	9		=	•	
11	5					11	5				
16	5					10	4				
20	12					16	6				
12	5					8	8	=		•	
10	14		−4		−4	13	6				
14	9	5				3	8		−5		−3.5
9	3					14	2				
19	10					7	6	1		1.5	
17	7					6	7		−1		−1.5
10	8	2		3		13	8	5		3.5	
17	15	2		2		14	2				
27	26	1		1		42	19				
T: 252	162					237	172				
% 156	100					138	100				
T of negative rank values:				−4.0						−5.0	
N:				15						13	
P =				<0.01						<0.01	

Note: T = totals; N = number of paired comparisons; P = probability.

It is not necessary to record all the differences. The negative differences are recorded first; then only those positive differences which are smaller or equivalent. Since the totals of negative rank values determine P in Wilcoxon's tables, higher positive values are of no interest in a simple test of significance. Because of two equal counts in Comparison No. 2, the actual number of paired determinations is reduced to 13.

merating organisms. The filming of 1 per cent serum containing dilute india ink (6) or blood cells (21) is practiced until high reproducibility is evident. After fixation and staining, critical inspection and low-power examination reveal whether perfect films have been retained.

Expedients which facilitate counting and analyses may be summarized as follows. The microscopic field is divided into 36 squares by means of a Howard mold counting disc (Bausch and Lomb No. 31-16-15) in the ocular of the microscope. A flat field is assured by restricting all observations to the central 16 squares. Within this area, 4 horizontally arranged squares are regarded as a unit or "block." Observation of the frequency of microorganisms at each margin of a film (table 3) predicts whether one, 2, 3, or 4 blocks should be examined in order to observe 140 or more bacilli while crossing the diameter of each film. Although bacilli are totaled on a counter, the number observed in each individual "field" (table 4) is recorded while examining one continuous strip of 15 fields across the diameter of each film. Having accumulated 15 measurements of the concentrations of bacilli per film, advantage is taken of the paired replicate analyses of Wilcoxon (22) to ascertain if the results in films prepared from different samples differ significantly. This work usually can be restricted to two films of ideal character and size from each sample; further counts are made if there are discrepancies between two counts from a given sample. The type of table employed for entry of these data and for analyses is shown in table 4, which sets forth comparisons of the relative numbers of *M. lepraemurium* observed in control and tissue-cultured samples as recorded by two observers.

During routine use of this method, but one source of error has been discovered which is recognized readily by inspection of tabulated counts per field. Concentrates of bacilli released from dense cell suspensions may produce elevated concentrations of bacilli near the centers of films. This phenomenon may increase the total bacilli recorded per strip by as much as 20 per cent. The incorporation of 0.05 per cent bile during homogenization (5) has avoided this difficulty.

Discussion

In this laboratory and elsewhere studies on the fate of mycobacteria in cell and tissue cultures have been based on two natural assumptions: that difficulties due to the focal character of mycobacterial infections in biopsied tissues might be avoided if known numbers of cells residing on glass were infected with known numbers of bacilli, and that the resultant, more standardized systems should be susceptible to direct microscopic analysis of the fate of mycobacteria within cells. Continual study of the problem of measuring small changes in bacterial populations within cells has emphasized certain fallacies in these assumptions. Wide variations in initial bacterial content arise from the different phagocytic activities of individual cells. These initial values are distorted by growth or decline of either the cell or the bacillary populations. Careful efforts to enumerate bacilli per cell and to multiply by cell numbers are complicated by the necessity for practical estimations of bacillary content, by irregular distributions of cells, by the loss of

the more heavily loaded cells, and sometimes by the desirability of evaluating modest alterations in the length of mycobacterial units.

In view of certain considerations yet to be assembled, it may be suggested that direct microscopic examination of bacilli within cells should be reserved for cytologic study or for circumstances in which losses of heavily bacillated cells can be ignored and growth or destruction of microorganisms is so clear cut as to warrant forthright conclusions. In such instances, a magnification of 100 times facilitates counting total cells per unit area of coverslip or loop-droplet of suspension while recording observations in three classes: no bacilli seen, low-power positive, and "stuffed" cells.

When it is necessary to deal with more quantitative issues, e.g., the difference between cells possessing different levels of resistance or differences due to slow growth or destruction of bacilli, basic difficulties arise. The anticipation has been that recent advances in the manipulation and enumeration of cells, together with quantitated infections, would permit definitive study of host-parasite relationships. The fact is that organisms as supposedly nontoxic as human or murine leprosy bacilli cause preferential release and degeneration of infected monocytes and fibrocytes. The degeneration of cells infected with tubercle bacilli has been emphasized repeatedly (4, 16, 23–25) and employed as the basis of various arguments. Nevertheless, there are no data in this laboratory or in published works which reveal the number or proportion of cells and mycobacteria which have eluded observation. It seems futile to undertake to plate, count, or calculate in both coverslips and in successive supernates the numbers of bacilli retained in surviving and degenerating cells and occurring free in the medium. The present suggestion that all accumulated supernates and rinsings must be combined with the bacilli from disrupted cells, and that total counts must be undertaken, is but a stop-gap. Although it assures that some important segment of the mycobacterial populations will not escape consideration, the mycobacteria concerned have escaped the dynamic relationships proposed for investigation.

Summary

Although reproducible infections are established by means of pipetted aliquots of cells and bacilli, the initial distributions of bacilli among the cells are not adapted to microscopic assay of moderate growth or destruction of the bacillary populations. The original percentages of cells with bacilli, or numbers of bacilli per cell, are modified by growth or deterioration of cells as readily as by a rise or decline in bacterial numbers. Losses of the more heavily infected cells from glass during replacement of supernates and moderate changes in the length of bacterial units complicate further any attempts to base quantitative assays on the mycobacterial content of cells.

Simplified methods of estimating total cells and gross changes in mycobacterial populations by means of low-power examination have been suggested. For more quantitative purposes, total plate or microscopic counts of the mycobacteria liberated into supernates and from disrupted cells are required. A method has

been described for judging the significance of differences in microscopic counts without accumulating a series of totals from each preparation.

Sumario

Valoración del Destino de las Micobacterias en los Cultivos de Células y Tejidos

Aunque se establecen infecciones reproducibles por medio de partes alicuotas de células y bacilos, vertidas con pipetas, las distribuciones iniciales de los bacilos entre las células no se prestan para la valoración microscópica de la proliferación o destrucción moderadas de las colonias bacterianas. Los porcentajes primitivos de células con bacilos, o de la cantidad de bacilos por célula, se ven modificados por la proliferación o el deterioro de las células con la misma facilidad que por el aumento o la baja del número de bacterias. Las pérdidas de las células más infectadas del vidrio durante la substitución de las sobrenadantes y las moderadas alteraciones del largo de las unidades bacterianas complican aun más todo esfuerzo encaminado a basar las valoraciones cuantitativas en el contenido micobacteriano de las células.

Se han propuesto técnicas simplificadas para calcular el total de células y las alteraciones macroscópicas de las colonias micobacterianas por medio del examen a poco aumento. Para fines más cuantitativos, se requieren numeraciones totales microscópicas o en placas de las micobacterias lanzadas a las células sobrenadantes o procedentes de las células desintegradas. Se describe un método dedicado a juzgar la importancia de las diferencias en las numeraciones microscópicas sin acumular una serie de totales para cada preparación.

Resume

Détermination du sort des mycobactéries dans les cultures de tissus

Bien que les infections reproduisibles soient déterminées par pipettage d'aliquotes de cellules et de bacilles, les répartitions initiales de bacilles parmi les cellules modéré ne se prêtent pas à la détermination microscopique du développement ou de la destruction modérés des populations bacillaires. Les pourcentages originaux de cellules avec bacilles, ou du nombre de bacilles par cellules, sont modifiés par le développement ou l'altération des cellules, autant que par l'élévation ou la réduction du nombre des bactéries. La perte à la surface duverre des cellules les plus infectées, lors du remplacement de la couche superficielle du milieu et d'une modification de la taille des unités microbiennes compliquent encore toute tentative d'évaluation quantitative basée sur le contenu bactérien des cellules.

L'auteur propose des méthodes simplifiées permettant d'évaluer à l'aide de faibles grossissements le nombre global des cellules et les altérations macroscopiques survenues dans la population mycobactérienne. Quant il s'agit d'évaluations quantitatives, les numérations microscopiques globales des mycobactéries dans les boîtes de Pétri ou libérées dans la couche surnageante et de celles provenant de cellules éclatées sont nécessaires. Une méthode permettant d'évaluer les différences observées dans les numérations microscopiques, sans recourir à l'accumulation d'une série de totaux pour chaque préparation est décrite.

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THE EFFECTS OF EXTENDED INCUBATION ON PROPAGABILITY OF TUBERCLE BACILLI[1, 2]

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INTRODUCTION

An index of infectiousness is usually correlated with the propagability of tubercle bacilli contained in exudates from tuberculous patients. Cultural methods have also become a criterion for evaluating chemotherapeutic regimens. Prior to the drug era, specimens were assumed to be negative for viable tubercle bacilli if the organisms did not grow within six to eight weeks after inoculation of the specimen onto a suitable medium. The "smear-positive and culture-negative" reports, now seen with considerable frequency, were then a rare observation.

This nonculturable status of visible acid-fast bacilli from resected specimens has demanded the attention of several investigators, and the value of extended incubation in reducing the total number of these controversial microbes has been demonstrated (1–4). Vandiviere and associates (1, 3, 4) used extended incubation alone, whereas Hobby and her associates (2) devised new measures and elaborated standard techniques with the extension of incubation time.

Late emergence has been observed most frequently from resected closed cavities (3), and explanations have been offered which suggest factors of bacterial metabolism, physicochemical and chemotherapeutic effects. Presumably bacilli in sputum may be shed from open cavities, endobronchial disease, or tubercles, tuberculomas, and closed cavities which may microscopically or macroscopically erode and emit bacilli into the bronchi. It is generally accepted that most bacilli are shed from open cavities and these are generally "fast-growing" bacilli, but it must be recognized that bacilli may arise from these other sources, and it is not feasible to foretell their origin. In view of these considerations, prior explanations of delayed growth may well be applicable to the data reported herein.

Several investigators (5–8) have suggested that occurrence of positive smears and negative cultures and late emerging tubercle bacilli may be due to retention of a sufficient amount of para-aminosalicylic acid (PAS) in the oral cavity or the sputum collection bottle to prevent or delay growth of microörganisms which are susceptible to this drug. Jones and Gentry (9) have suggested that the addition of isoniazid to drug regimens may be responsible for the retardation of growth of certain cultures. It has been stated by Duerr and Black (10) that

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isoniazid has a suppressive effect, but that this is less than that of PAS. Stewart and co-workers (11) have minimized the importance of the late emergence of tubercle bacilli. Howard and associates (12) have observed no particular effect from any specific drug or combination of drugs. They have reported that late emergence is of little importance because, in their study, none of the cultures which required prolonged incubation were of diagnostic value. Also, their studies of drug susceptibility on cultures which grew within a normal incubation period agreed with those of slow-growing bacilli from the same patients.

The present study was prompted by several observations from nonoperative and operative material. They were (*1*) late emergence of growth from specimens (morning specimens only) of patients who were not receiving drugs in liquid form, (*2*) late emerging growth from nonsurgical patients from whom no positive cultures had been isolated by conventional incubation measures, and (*3*) growth of bacilli by extended incubation from resected material of patients revealing no other positive evidence of living acid-fast organisms. It is proposed, therefore, to endeavor to establish the significance of cultures of tubercle bacilli which emerge on prolonged incubation. How do they differ from their predecessors and what is their importance in evaluating the infectiousness of their hosts?

Methods

Specimens to be cultured for tubercle bacilli were inoculated onto Löwenstein-Jensen medium contained in 23 by 85 mm. culture tubes tightly sealed with nontoxic, rubber-lined screw caps.[3] The medium in a few of the tubes dried out before the end of the prolonged incubation period. The tubes contained sufficient oxygen for good growth. Those cultures which revealed no visible growth in eight weeks were reported as being negative. However, incubation of these was continued, and they were subjected to examination at monthly intervals. Those negative after the eighth month of incubation were discarded. The 91 positive cultures thus obtained were designated as "late growers" and are the subject of this study. Late-emerging colonies of acid-fast bacilli were subcultured at the time of their discovery to the medium of Löwenstein-Jensen, and the time required for visible growth to develop was again observed. Subcultured growth was used for inocula for drug-susceptibility studies, tests for catalase activity, and determination of virulence for guinea pigs. Susceptibility tests were made with the incorporation of the drugs in Löwenstein-Jensen medium (13).

Inocula for drug-susceptibility tests consisted of 0.1 ml. of an even saline suspension of the subcultured growth (14). A definition of drug concentration to which growth was considered a manifestation of resistance is described elsewhere (4), wherein growth in the presence of 10 γ of streptomycin, 10 γ of PAS, and 5 γ of isoniazid per ml. of medium indicated resistance. Also, evaluations of significance were made with growth in the presence of 100 γ, 50 γ, and 10 γ, respectively, denoting resistance. These were not considered to imply clinical significance but to denote changes in bacterial populations. Animals were inoculated by subcutaneous injection in the inguinal region and were sacrificed after eight weeks. Gross evidence of infection of the viscera was noted, and acid-fast smears were made of the common-iliac and tracheobronchial nodes. Tissues from these nodes were cultured from animals whose organs were negative microscopically for acid-fast bacilli.

Results

A tabulation of the results of cultures planted for acid-fast bacilli over a period of four years is presented in table 1. The total number of specimens containing

[3] Caps supplied by Armstrong Cork and Closure.

TABLE 1

RESULTS OF CULTURES INOCULATED FOR TUBERCLE BACILLI

Cultures	Number	Per Cent
Total number of specimens cultured	8,165	100.0
Negative (8 weeks' incubation)	6,076	74.4
Positive (8 weeks' incubation)	1,725	21.1
Unsatisfactory	364	4.5
Positive on extended incubation	91	
Per cent of negatives at 8 weeks		1.5
Per cent of all positive cultures		5.0
Per cent of all satisfactory cultures		1.2
Per cent of all cultures		1.1

bacilli capable of producing visible colonies in the conventional (eight weeks) incubation period is shown, along with the number and per cent which required extended incubation for growth. From 8,165 specimens cultured, 1,725 (21 per cent) were positive in eight weeks or less, 364 (4 per cent) were unsatisfactory, and of the remaining 6,076 (75 per cent), 91 (1.1 per cent) of all specimens, or 1.5 per cent of negative cultures) produced growth on prolonged incubation. The 91 late-growing bacilli were isolated from 51 patients. Thirty of these cases revealed only one late-emerging culture while the remaining 21 patients had from two to ten retarded cultures. In a few cases, transplants of the late cultures failed to grow, and in each instance this was attributed to excessive dryness. Generally, subcultures grew within the eight-week incubation period. There were only two instances in which subcultures grew after the eighth week of incubation. Both of these subcultures came from patients who produced other late-growing bacilli that emerged in the conventional time upon subculture.

A breakdown of the data on patients who had late cultures positive for tubercle bacilli is presented in table 2. The total number which were positive in the conventional time and on extended incubation is shown, along with the per cent of all positive specimens which revealed retarded growth. Patients from whom no conventional positive cultures originated are categorized separately from those who revealed such growth. An average of approximately three retarded cultures was observed among the patients from whom more than one "late-grower" was isolated. Ten such cultures grew from specimens from one patient who, nevertheless, had five positive cultures by conventional incubation. As many as five "late-growers" were isolated from several patients. Bacterial colonies emerged by extended incubation of cultures of specimens from 6 patients who had not been proved positive by growth in the usual incubation time. Four of the patients revealed only one late culture each, and the other 2 had three and five retarded "growers," respectively. A smear positive for tubercle bacilli was obtained from only 2 of the patients represented by these cultures.

In table 3 may be seen the duration of various drug regimens prior to the isolation of the late-growing strain of tubercle bacilli from each patient. The data are grouped according to whether the patient had only late positive growers

TABLE 2

RELATION OF NUMBERS OF POSITIVE CULTURES IN CONVENTIONAL AND EXTENDED INCUBATION PERIODS FROM INDIVIDUAL PATIENTS HAVING LATE-GROWING TUBERCLE BACILLI

Bacteriology of Patients	Number of Conventional Cultures	Number of Late-growing Cultures	Per Cent of Late-growing Positive Cultures
Patients who produced positive cultures only by extended incubation. These late growers were diagnostic.	0	1	100
	0	1	100
	0	1	100
	0	1	100
	0	3	100
	0	5	100
Patients from whom positive cultures were isolated by conventional incubation measures and who also had late growers. A breakdown comparing the per cent of late-growing cultures between patients who had only one retarded culture and those who had more than one does not indicate a trend in the direction of predominance of late growers from those who had such cultures repeatedly.	1	2	67
	1	2	67
	1	2	67
	5	10	67
	2	2	50
	1	1	50
	1	1	50
	1	1	50
	3	2	40
	8	5	38
	4	2	33
	2	1	33
	2	1	33
	2	1	33
	7	3	30
	5	2	29
	13	5	27
	6	2	25
	3	1	25
	3	1	25
	3	1	25
	7	2	22
	8	2	20
	4	1	20
	4	1	20
	4	1	20
	4	1	20
	5	1	17
	5	1	17
	11	2	15
	6	1	14
	6	1	14
	6	1	14
	6	1	14
	15	2	12
	17	2	11
	19	2	10
	9	1	10
	10	1	9
	10	1	9
	10	1	9
	11	1	8
	31	2	6
	19	1	5
	22	1	4
Total	(323)	(91)	(28) (average)

TABLE 3

MONTH'S DURATION OF VARIOUS DRUG REGIMENS PRIOR TO THE ISOLATION OF THE FIRST LATE-GROWING TUBERCLE BACILLI FROM EACH PATIENT

Patient Group	Streptomycin	PAS	Isoniazid
I. Patients who produced positive cultures only by extended incubation.	36	36	24
	14	14	—
	9	9	—
	24	24	—
	—	10	10
	—	—	6
Average months per patient:	(21)	(19)	(13)
II. Patients who produced only one late grower, but who had other conventional positive cultures.	24	3	24
	1	1	3
	16	4	20
	25	13	33
	6	5	11
	6	8	8
	1	1	2
	31	12	18
	24	2	2
	10	19	9
	21	27	27
	2	31	25
	3	7	7
	2	5	5
	16	16	3
	5	5	1
	25	25	25
	6	12	12
	14	14	—
	1	—	1
	6	—	6
	20	—	20
	—	4	4
	—	7	7
	—	5	5
	—	2	2
Average months per patient:	(12)	(10)	(11)
III. Patients who produced two or more late growers and also had other conventional positive cultures. The average duration of drug therapy in this group is significantly higher than that in *Group II*.	13	17	17
	12	12	12
	29	23	17
	23	25	26
	5	20	26
	2	12	12
	28	28	23
	30	20	38
	60	60	60
	28	39	18
	5	5	16
	9	11	2
	48	22	48
	34	34	—
	32	—	32
	—	19	19
	—	5	5
	—	—	6
	—	—	—
Average months per patient:	(24)	(22)	(22)

or both these and conventional cultures and whether one or more than one "late-grower" was shed. Most of the patients were treated continuously or periodically with multiple regimens, including streptomycin, isoniazid, and PAS, prior to the isolation of the late-growing strain of tubercle bacilli. The over-all duration of drug therapy is considered, rather than just the duration of therapy of those drugs being received at the time the specimen was taken. The average period of this drug treatment was approximately twice as long (twenty-three months) for those who repeatedly shed slow-growing bacilli as for those who had only one such culture. One patient, from whom two positive cultures were obtained by extended incubation, had received no drugs previously. Two other patients, one having two "late-growers" and the other five, revealed these cultures after only six months of isoniazid therapy. In most cases, however, repeated late-growing tubercle bacilli were observed among patients who had received a drug or combination of drugs for periods of twelve months or longer. There were only four exceptions to this observation. Fifteen similar exceptions were observed in the data from patients having only one "late-grower." Seven patients who shed slow-growing bacilli had not received PAS. Five of these received isoniazid alone or along with streptomycin. Six patients who produced late-growing tubercle bacilli had not received isoniazid, and 10 such patients had had no streptomycin therapy.

Additional studies were made with transplants of cultures from 29 of the 51 patients who shed late-growing bacilli, and these more detailed investigations are reported in table 4.

Drug-susceptibility data were available on acid-fast bacilli which grew in the conventional incubation period from 26 of these patients. These data were compared with susceptibility results of the corresponding "late-growers" from the same patients, as shown in table 4. In cases in which susceptibility data were available on more than one regular culture, the one planted closest to the date of the "late-grower" was used.

Of the 29 late-growing cultures of tubercle bacilli studied, 14 were resistant to PAS and/or isoniazid, depending on whether the patient had received one or both of these drugs. Comparisons with previous drug-susceptibility studies indicate a difference in susceptibility to at least one of the three drugs between the regular cultures and the same patient's "late-growers" in 8 of 26 (31 per cent) cases tested or in 10 of 78 (13 per cent) individual drug-susceptibility tests. Of those which disagreed, five were susceptibility tests to PAS, four to isoniazid, and one to streptomycin. No correlation was indicated between disagreement and duration of chemotherapy or the sputum content of tubercle bacilli prior to the isolation of the "late-grower."

Results of animal studies with "late-growers" are presented in table 4. Only 15 of the 17 isoniazid-resistant strains of late-growing bacilli were catalase negative. All but two (both catalase negative) produced progressive disease in animals, a point which suggests a difference between "late-growers" and the conventional population of tubercle bacilli. Three morphologically atypical "late-growers" were

TABLE 4

STUDIES OF TUBERCLE BACILLI CULTURES WHICH REQUIRED EXTENDED INCUBATION FOR GROWTH

Number of Patients	Colony Morphology	Susceptibility Data			Catalase Activity	Virulence in Animals
		Streptomycin	PAS	Isoniazid		
		(Resistant to Isoniazid)				
1	T	S/S	S/R	R/R	−	+
2	T	S/S	S/S	R/R	−	+
3	T	R/R	S/S	R/R	−	+
4	T	S/S	S/S	R/R	−	+
5	T	R/R	R/R	R/R	−	+
6	T	S/S	R/R	S/R	−	−
7	A	/R	/R	/R	+	+
8	T	S/S	R/R	R/R	−	+
9	T	S/S	S/R	S/R	−	+
10	T	S/S	S/S	R/R	−	−
11	T	R/R	R/R	R/R	−	+
12	A	S/S	R/R	R/R	+	+
13	T	S/S	R/R	S/R	−	+
14	T	R/R	R/R	R/R	−	+
15	A	S/S	S/S	R/R	−	+
16	T	S/S	S/S	R/R	−	+
17	T	S/S	S/R	S/R	−	+
18	T	S/S	S/S	R/R	−	+
		(Susceptible to Isoniazid)				
1	T	S/R	S/S	S/S	+	+[d]
2	T	/S	/S	/S	+	+
3	T	S/S	S/S	S/S	+	+
4	T	S/S	S/S	S/S	+	+[d]
5	T	S/S	S/S	S/S	+	+[d]
6	T	S/S	S/R	S/S	+	+[d]
7	T	S/S	S/S	S/S	−	+[d]
8	T	S/S	S/S	S/S	+	+
9	T	S/S	S/S	S/S	+	+
10	T	/R	/R	/S	+	+
11	T	S/S	S/R	S/S	+	+[d]

T—typical colonies; A—atypical colonies; S—susceptible; R—resistant; S/R—conventionally incubated culture susceptible/same patient's late culture resistant; S/S—conventionally incubated culture susceptible/same patient's late culture susceptible; R/S—conventionally incubated culture resistant/same patient's late culture susceptible; R/R—conventionally incubated culture resistant/same patient's late culture resistant; +—catalase present, virulence; +[d]—culture virulent and animal died of tuberculosis prior to eight weeks following injection.

studied, all of which were isoniazid resistant and pathogenic for guinea pigs; however, two of them were catalase positive. The very tiny size of the colonial forms of these two strains was the only atypical manifestation; they were rough colonies and of normal color. Four patients who shed isoniazid-resistant

"late-growers" had isoniazid-susceptible cultures isolated by conventional incubation. None could be considered as true atypicals (15) although they were most certainly different populations from the classical human organisms.

Also included in table 4 are data on 11 isoniazid-susceptible, late-emerging cultures of mycobacteria. One of these was catalase negative, although highly infectious for guinea pigs. There was complete agreement in isoniazid-susceptibility results of conventional and late cultures in this group, and no morphologically atypical types were observed. Gross evidence and progression of disease occurred to a greater extent among this group of "late-growers" than among the isoniazid-resistant group.

Results of animal inoculations, drug susceptibility studies, and catalase activity tests were similar with "late-growers" from patients who shed them repeatedly and with cultures which represented the only late growth from a patient

Discussion

In some laboratories the attitude is taken that the incidence of late-growing tubercle bacilli is not sufficiently great to justify extended incubation of cultures. The occurrence of "late-growers" is attributed to the use of drugs, and late emergence is not questioned further. A first glance at the results in the present study might support such a point of view. The over-all gain of 1.1 per cent in positive growth by extended incubation hardly seems great enough to necessitate crowding incubators and using personnel time to regularly check a large bulk of negative cultures. The fact that about 60 per cent of the patients from whom "late-growers" were isolated had only one such culture and that most of these patients had other cultures positive for tubercle bacilli by conventional (eight weeks) incubation might support this attitude. Many "late-growers" followed a period of easy demonstration of tubercle bacilli in the sputum and represented a stage when reversal of infectiousness was taking place; and in some cases an isolated late colony appeared among many conventional cultures. These single "late-growers," thus, would seem to be of minimal importance and, in the latter case, merely a matter of chance, technique, or other uncontrolled factors. Nevertheless, these were virulent tubercle bacilli and repeatedly occurred in many patients who would have been assumed to have noninfectious sputum if the "late-growers" had not been detected. Such cultures were of diagnostic value in 6 cases in the present study, as they represented the only positive bacteriologic evidence of tuberculosis in these patients. A total of 63 patients having pulmonary involvement, who were admitted to this diagnostic and referral hospital during the four-year study, had no definite diagnosis by biochemical, pathologic, or bacteriologic studies. Late-growing mycobacteria were obtained from 6 of these patients and thus reduced the number of undiagnosed cases by about 10 per cent. Therefore, extended incubation measures resulted in 10 per cent fewer undiagnosed cases than would be expected by incubating cultures for only eight weeks.

The cumulative duration of chemotherapy for each patient has been considered instead of the duration of the particular drug treatment in use at the time of the revelation of retarded bacilli. It is recognized that cumulative chemotherapy is not a favorable approach to demonstrate an effect such as drug concentration in

the sputum. Yet, this approach is fundamental if chemotherapeutic effects on the patients' changing population of bacilli are to be studied.

A relationship is indicated between late emergence of acid-fast cultures and the duration of chemotherapy. The data presented suggest that late-growing bacilli were isolated most frequently from patients who had been on chemotherapeutic agents for at least twelve months, and the repeated occurrence of retarded growth from the same patients was more prevalent from those on a longer term of drug therapy. Some conflicting evidence was observed, however, in that one patient who had had no drugs shed two late-growing strains of tubercle bacilli, both of which were virulent. Two other patients revealed two and five late-growing strains, respectively, after only six-months' administration of isoniazid, and still another patient had one late-growing culture after two months of isoniazid-PAS. Such an observation might be explained as having been brought about partly by long exposure of the microorganism to the natural defense mechanisms of the human body. These cases, however, are exceptions and, in spite of their existence, the data still suggest a trend of delayed growth attributed to long-term drug treatment. Thus, late-growing cultures might be indirectly attributed to chemotherapeutic effects on the genetic or metabolic patterns of the microorganism.

The slow-growth characteristics may be representative of an inter-organism alteration, for which the drug is the initiating factor, but this correlation with therapy does not necessarily suggest the presence of inhibiting concentrations of drug in the exudate specimen. It appears unlikely that late emergence, as observed in the present study, was due to drug concentration in the sputum because a majority of the microorganisms so isolated were drug resistant. Moreover, they were grown from morning sputum specimens wherein there was no opportunity for prolonged drug contact in the specimen bottle. It is difficult to align late emergence with the administration of a particular drug, since most of the patients were treated with multiple-drug regimens, including streptomycin, PAS, and isoniazid. But in 7 patients included in this study, late emergence could not be associated with PAS therapy, as the patients had not received this drug. Six patients had not been treated with isoniazid, but all patients having late-growing strains of bacilli, except one, had received either or both PAS and isoniazid. Thus, all patients but one had taken an orally administered drug, but none were given the drug in liquid form (7).

In this study, the ten incidents of disagreement of drug susceptibility data between "late-growers" and the same patient's conventional cultures were all due to drug resistance of the slow-growing bacilli. These observations were made considering growth in the presence of 10γ of streptomycin, 10γ of PAS, and 5γ of isoniazid per ml. of medium as manifestations of resistance. It is recognized that many people prefer to refer to growth in the presence of these drug concentrations as indicating partial resistance, and to use growth in the presence of 100γ of streptomycin, 50γ of PAS, and 10γ of isoniazid as the criterion for resistance. If these standards had been used in the present study, in only 9 instances would the term "partial resistance" have been used in place of "resistance." All 9 of these instances were concerned with susceptibility to streptomycin and PAS

and none to isoniazid. Therefore, by either standard, the results remain essentially the same. The frequency of resistance of "late-growers" was noticeable—especially to isoniazid. Normal resistance of the patient host may be responsible for the decrease in rate of metabolism, and it is possible that isoniazid resistance which has been associated with the absence of catalase activity and attenuation of virulence for laboratory animals (16) is not necessarily (but may be) associated with a very slow metabolism rate which makes the bacilli require a longer incubation period. However, most of the isoniazid-resistant organisms included in this study caused progressive disease in animals and, although virulent, the degree of gross evidence of infection was low. The lack of ability of viable tubercle bacilli to produce visible colonies in eight weeks may be on occasion obscurely associated with isoniazid resistance, absence of catalase activity, lowered virulence for guinea pigs, or factors at present poorly understood.

Late-growing tubercle bacilli from resected tissue have been reported as generally emerging from remote lesions (3), but the origin of these slow growers in sputum is unknown. Therefore, the efficacy of continued chemotherapy in such instances remains unanswered by these observations.

Are "late-growers" variants or mutated strains of *M. tuberculosis*? In studies of this sort it must be recognized that what is tested in a subculture may not correspond directly to what was obtained on primary culture. Thus, it can only be said that they do not react to human manipulations as their immediate parents, but neither do they react as the earlier ancestors of their parents. They behave as a new culture only influenced by their heredity. Reactions in response different from what would be expected from conventional pathogenic acid-fast strains were observed and do suggest changes. Two morphologically atypical "late-growers" were isoniazid resistant; yet they showed catalase activity and virulence for laboratory animals. The cultures morphologically typical for *M. tuberculosis* which were isoniazid-resistant differed from most isoniazid-resistant organisms as reported in the literature (17) in that all but two of them caused progressive disease in guinea pigs. Isoniazid-susceptible organisms appeared as typical acid-fast cultures, except for their retarded rate of growth. Only one exception was observed and that one was catalase negative although the animal injected with it died of tuberculosis in less than eight weeks. However, the question of mutated strains remains unanswered.

Prolonged incubation is not necessary for the activation of transplants of late emerging cultures. Virulence studies show that their hosts are infectious, and the danger of promiscuous spread from unrecognized carriers must be realized. In the present study, ten "late-growers" and five conventional growers originated from one patient who had been on streptomycin, PAS, and isonazid for about five years prior to isolation of the first "late-grower." These ten "late-growers" were isolated over a period of four months. Five of them came from consecutive specimens obtained prior to surgical procedures and two of these were taken immediately before the operation. By conventional measures, results of all specimens indicated sputum negative for tubercle bacilli at the time of the patient's operation. Subsequently, it was learned by extended incubation some time after this patient

had undergone surgery that his sputum had contained tubercle bacilli at the time of the operation and this had continued postoperatively. Four additional late-growing cultures of tubercle bacilli came from immediate postoperative sputum specimens, all of which had been negative on microscopy; another occurred approximately four months later. This kind of postoperative observation might be expected on the basis of previous work (3) which indicated that the cultivation of tubercle bacilli from resected closed cavities usually requires prolonged incubation. This information is of value from the standpoint of the course of action of the physician and the patient's welfare, as well as his state of infectiousness to others. Extended incubation with late data is obviously not the answer. A more practicable approach is needed in order to avoid such situations.

Summary

A total of 91 late-growing cultures of tubercle bacilli isolated from 51 patients were studied. Six of these cultures were of diagnostic value and represented about 10 per cent of cases previously undiagnosed by biochemical, pathologic, or bacteriologic studies. The late cultures positive for tubercle bacilli resulted in a gain of 1.1 per cent. On the average, patients who produced "late-growers" repeatedly had been on chemotherapy about twice as long as those from whom only one "late-grower" was isolated.

The "late-growers" did not require extended incubation to grow from subcultures. They were found to be predominantly isoniazid resistant, and as such were generally negative for catalase activity but did produce progressive disease in guinea pigs. The isoniazid-susceptible "late-growers" behaved very much like conventional tubercle bacilli. It was concluded that tubercle bacilli which require extended incubation for growth are of value for diagnostic purposes and in evaluating the state of infectiousness of their hosts, and the frequency of such cultures probably justifies prolonged incubation in situations in which facilities permit.

Sumario

Los Efectos de la Incubación Prolongada sobre la Propagabilidad de los Bacilos Tuberculosos

Se estudió un total de 51 cultivos de crecimiento tardío, aislados de 51 enfermos. Seis de estos cultivos poseían valor diagnóstico y representaban aproximadamente 10 por ciento de los casos que no se habían diagnosticado antes con estudios bioquímicos, histopatológicos o bacteriológicos. Los cultivos tardíos positivos para bacilos tuberculosos dieron por resultado un aumento de 1.1 por ciento en la positividad. En conjunto, los enfermos que produjeron repetidamente "crecedores tardíos" habían estado a quimioterapia aproximadamente dos veces más tiempo que aquéllos de los cuales no se aisló más que un "crecedor tardío."

Los "crecedores tardíos" no requirieron incubación prolongada para desarrollarse de subcultivos. Según se observó, eran predominantemente isoniacido-resistentes, y en esa capacidad se mostraron generalmente negativos en cuanto a actividad catalásica, pero sí produjeron enfermedad evolutiva en los cobayos. Los "crecedores tardíos" isoniacido-susceptibles se comportaron en forma muy semejante a los cultivos de bacilos tuberculosos convencionales. Se dedujo que los bacilos tuberculosos que requieren incubación prolongada para formar colonias son de valor para fines diagnósticos y para justipreciar el estado de infecciosidad de los huéspedes y que la frecuencia de esos cultivos probablemente justifica la incubación prolongada en situaciones en que lo permiten los medios disponibles.

Resume

Les effets de l'incubation prolongée sur le pouvoir de propagation des bacilles tuberculeux

Au total, 91 cultures à développement tardif provenant de 51 malades ont été étudiées. Six de ces cultures avaient une valeur diagnostique et représentaient environ 10% de cas où les études biochimiques, histopathologiques ou bactériologiques n'avaient pas permis le diagnostic. Les cultures à développement tardif, qui étaient positives pour le bacille tuberculeux présentaient un gain de 1.1%. D'une manière générale, les cas où se produisaient constamment des colonies tardives (late growers) se manifestaient chez des malades qui avaient été soumis à la chimiothérapie au moins deux fois plus longtemps que ceux où il n'a été isolé qu'une seule "colonie tardive."

Les "colonies tardives" ne réclamaient pas l'incubation prolongée pour pousser à partir de sous-cultures. On observait qu'elles étaient de façon prédominante résistante à l'isoniazide, et à ce titre la réaction de la catalase a été généralement négative; néeanmoins, elles déterminaient la maladie évolutive chez le cobaye. Les "colonies tardives" isoniazidosensibles se comportaient à peu près comme les cultures ordinaires des bacilles tuberculeux. En conclusion, les bacilles tuberculeux, réclamant l'incubation prolongée pour se développer sont utiles dans le diagnostic et l'évaluation du degré d'infectiosité de l'hôte. D'autre part, la fréquence de ces cultures justifie probablement l'incubation prolongée dans les circonstances où elles sont praticables.

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THE EFFECT OF CHEMOTHERAPY ON THE LEUKOCYTIC SENSITIVITY TO TUBERCULIN IN GUINEA PIGS EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM TUBERCULOSIS H37RV[1, 2]

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INTRODUCTION

In a previous study (1), the writers found that 79 per cent of a group of subjects with inactive tuberculosis failed to show leukocytic sensitivity to tuberculin when tested by a tissue culture technique. Some of these subjects had been considered to have had inactive tuberculosis for many years, but most of them had been released from a tuberculosis sanatorium within a year. All of the subjects that were tested (about 50 per cent) gave positive tuberculin skin test reactions. Since the tissue culture technique had been found to be just as capable of detecting tuberculin sensitivity as the intradermal skin test (2), it seemed probable that differences in the sensitivities of the skin and leukocytes, observed in the human subjects with inactive tuberculosis, were due to a loss of leukocytic sensitivity. It appeared that one method of ascertaining whether this was so was to determine whether a similar loss or decrease in leukocytic sensitivity would occur in tuberculous animals as they responded to treatment. This paper describes the results obtained with experiments designed to show the effect of chemotherapy on the sensitivity of the leukocytes of tuberculous guinea pigs.

MATERIALS AND METHODS

Experimental approach: A group of 14 tuberculous guinea pigs previously shown to have both skin and leukocytic sensitivity to tuberculin (2) was divided into two groups. One group (8 animals) received chemotherapy, the other group (6 animals) did not. The sensitivities of the skin and leukocytes of both groups, as well as of two similar groups of nontuberculous animals, were determined at various intervals.

Chemotherapy: The chemotherapeutic agents and respective doses used were streptomycin sulfate (Abbott), 10 γ per kg. of body weight twice daily, and isoniazid, 15 mg. daily. Therapy was initiated eight weeks after infection and was continued for a period of ten weeks. Four of the animals received a second course of therapy beginning the twenty-first week after the termination of the first course.

Testing for tuberculin sensitivity: Skin sensitivity was determined by the intradermal injection of Old Tuberculin (OT) and leukocytic sensitivity by the tissue culture technique previously described (1). Whenever possible the animals were tested in groups of 4, consisting of one untreated normal animal, one treated normal animal, one untreated infected animal, and one treated infected animal. The degree of leukocytic sensitivity of the infected guinea pigs was determined by calculating the comparative cytotoxic index (CCI)[3] (3). Similar comparative cytotoxic indices were determined for the normal guinea

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[3] Comparative Cytotoxic Index (infected) $= \frac{\text{MD in OT (infected)}}{\text{MD in GB (infected)}} \times \frac{\text{MD in GB (normal)}}{\text{MD in OT (normal)}}$

pigs by calculating their cytotoxic indices[4] and dividing these by the mean cytotoxic index of the normal animals used in the tests of that day. The results for the 1:100 dilution are given in detail in table 1 and are summarized for all three dilutions in table 2.

Results

The results obtained with the two groups of uninfected animals (treated and untreated) were so similar that they were placed in a single group. Forty-one tests were made with the leukocytes of 13 different uninfected animals over a period of forty weeks with each of three dilutions of OT (1:100, 1:1,000 1:10,000). The range of the comparative cytotoxic index was from 0.70 to 1.30 with a mean ($\bar{X}$) of 1.00 and a standard deviation (S) of 0.14 for the 1:100 dilution; 0.86 to 1.16 with a mean of 1.00 and a standard deviation of 0.06 for the 1:1,000 dilution; and 0.76 to 1.20 with a mean of 1.00 and a standard deviation of 0.08 for the 1:10,000 dilution. Individual indices obtained with the leukocytes of infected animals which fell in the ranges below $\bar{X} - 2S$ were considered to be indicative of specific tuberculin sensitivity (0.72 for the 1:100 dilution, 0.88 for the 1:1,000 dilution, and 0.84 for the 1:10,000 dilution).

During the ten-week period of the first course of therapy, eighteen tests were made on the leukocytes of the uninfected animals with each of the three dilutions of OT and, similarly, sixteen tests were made on the leukocytes of the infected animals. The results obtained with the leukocytes of the infected animals, from the tests made during the second and fifth weeks after the initiation of therapy, were similar for all three dilutions of OT. The mean comparative cytotoxic indices of the infected animals were significantly lower than those of the uninfected controls, and all of the individual indices but one (0.95 with the 1:10,000 dilution, *No. 35*) were in the range of specific tuberculin sensitivity. By the ninth week after the initiation of therapy, however, the leukocytic sensitivities of the treated animals showed a significant change. The mean comparative cytotoxic indices had risen from 0.58 in the second week to 0.71 in the ninth week for the 1:100 dilution, from 0.69 to 1.00 for the 1:1,000, and from 0.76 to 0.98 for the 1:10,000, and the leukocytes of only 2 treated animals (*No. 4* at 1:10,000 and *No. 31* at 1:100) yielded indices in the range of specific tuberculin sensitivity.

During the entire period, the leukocytes of the untreated infected guinea pigs remained sensitive to tuberculin, and all of the infected animals gave positive tuberculin skin test reactions.

Since the number of animals used in the study was small, it seemed inadvisable to sacrifice any of them after the first course of therapy in order to determine whether or not infection still persisted. Instead, the animals were tested further at varied intervals during a period of twenty-one weeks to detect any changes in skin and leukocytic sensitivities.

[4] $\text{Cytotoxic Index (normal)} = \dfrac{\text{MD in OT}}{\text{MD in GB}}$

MD = migration distance
GB = glycerol broth
OT = Old Tuberculin

In the twelfth and fourteenth weeks after the initiation of therapy the mean comparative cytotoxic indices of the leukocytes from the infected guinea pigs were found to be relatively normal for all dilutions. The leukocytes of only one animal (*No. 15*) had indices indicative of sensitivity in all three dilutions (0.72, 0.54, and 0.60, respectively). By the eighteenth week, however, the mean comparative cytotoxic indices for the 1:100 dilution showed a significant drop to 0.74 and the leukocytes of 2 animals (*No. 7* and *No. 15*) had indices indicative of specific tuberculin sensitivity in all three dilutions. Further testing, in the twenty-third and twenty-eighth weeks, revealed that the leukocytes of 6 of the treated animals (*Nos. 1, 6, 7, 15, 23,* and *31*) had again become sensitive to two or more dilutions of OT while those of 2 of the animals (*Nos. 4* and *35*) had not.

In the thirty-first week, twenty-one weeks after the termination of the first course of therapy, a second ten-week course was initiated with 3 of the animals (*Nos. 1, 6,* and *31*) that had become leukocyte sensitive and one (*No. 35*) that had not. Skin and leukocyte sensitivity tests of all of the animals were made five weeks after initiation and at the termination of therapy. The animals were then sacrificed and necropsy was performed.

As was the case with the first course of therapy, the 3 animals that received the second course lost their leukocytic sensitivity but not their skin sensitivity while the untreated animal maintained both. With the second course of therapy, however, the loss of leukocytic sensitivity was detected after only five weeks as compared to nine weeks with the first course.

During the entire course of the study all of the infected animals gave positive skin test reactions to the 1:100 dilution of OT. One of the treated animals (*No. 7*) became negative to the 1:10,000 and 1:1,000 dilutions two and four weeks, respectively, after the termination of the first course of therapy. None of the controls gave positive skin reactions at any time, to any of the dilutions.

Cultural and histologic studies of the untreated infected animals that died or were sacrificed (*Nos. 2, 30, 32,* and *39*) revealed that all of them were tuberculous. Similar studies on the treated infected animals (*Nos. 1, 4, 6, 7, 15, 23, 31,* and *35*) revealed that animals *Nos. 15* and *23* were tuberculous. However, the remaining animals that received but one course of therapy (*Nos. 4* and *7*) as well as those that received both courses (*Nos. 1, 6, 31,* and *35*) were not actively tuberculous at the time of their death. This would indicate that at least 2 of the animals (*Nos. 4* and *7*) were probably cured by a single course of therapy. Of the other 6, one (*No. 35*) may have been cured by the first course while in 4 (*Nos. 1, 6, 15,* and *23*) the disease had become inactive but became active again in eight to twelve weeks. The second course of therapy appeared to have caused the disease to again become inactive.

An examination of the tabulated data reveals that there was considerable variation in the comparative cytotoxic indices of the nontuberculous animals. This was particularly true with the 1:100 dilution of tuberculin, in which the standard deviation was 0.1389. As has been previously observed (1), nonspecific factors appear to affect the migration of the leukocytes of nonsensitive subjects to a greater degree than they do those of sensitive subjects. This is borne out in

TABLE 1

SKIN TEST REACTIONS AND LEUKOCYTIC SENSITIVITIES (COMPARATIVE CYTOTOXIC INDICES) TO OLD TUBERCULIN, 1:100

Week after initiation of first therapy		2nd		5th		9th		12th		14th		18th		23rd		28th		36th		40th	
Week after initiation of second therapy																		5th		9th	
Group	*No.**	*S*†	*L*‡	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*
Uninfected animals treated and untreated	3			−	1.30	−	0.83					−	1.16	−	1.05	−	0.94			−	1.16
	17	−	1.10			−	0.94														
	18					−	0.94			−	1.00					−	1.08			−	0.88
	19			−	0.98																
	25			−	0.96																
	26	−	1.14			−	1.08														
	33			−	1.15	−	1.15					−	0.77								
	34			−	0.82			−	1.08			−	1.04	−	0.96						
	37			−	*0.70*			−	0.92												
	40	−	0.99			−	0.97			−	0.87	−	1.11					−	0.88	−	1.21
	41																	−	1.07	−	0.79
	42	−	0.81			−	1.04			−	1.14	−	1.04	−	1.00			−	1.06	−	0.94
	43					−	0.96														
Means			1.01		1.00		0.99		1.00		1.00		1.02		1.00		1.01		1.00		1.00

Infected animals treated once	1				+ 0.83		+ 0.76	+ *0.70*	+ 0.92	+ 0.83					
	4				+ 0.75			+ 0.75							
	6	+ *0.60*				+ 1.01	+ *0.67*	+ *0.67*	+ 0.75	+ 0.77					
	7		+ *0.69*			+ *0.72*	+ 0.88	+ *0.71*	+ *0.69*	+ 0.90					
	15		+ *0.58*			+ *0.72*	+ 0.86	+ *0.63*	+ 0.73				+ 0.78	+ *0.53*	
	23	+ *0.56*				+ 1.08	+ 1.10	+ 0.94	+ 0.83	+ 0.82			+ 0.92	+ *0.48*	
	31				+ *0.49*			+ 0.75	+ 0.82	+ *0.59*					
	35		+ *0.53*		+ 0.77			+ 0.80	+ 0.93						
Means		0.58		0.60		0.71	0.88	0.85	0.74	0.81		0.78	0.85		0.51
Infected animals treated twice	1												+ 0.87	+ 1.06	
	6												+ *0.71*	+ 0.99	
	31												+ 0.96	+ 1.00	
	35												+ 1.04	+ 0.93	
Means													0.98		1.00
Infected animals untreated	2		+ *0.54*		+ 0.73	+ *0.69*	+ *0.67*	+ *0.64*	+ *0.62*	+ *0.48*			+ *0.67*	+ *0.50*	
	30	+ *0.66*	+ *0.66*		+ *0.66*	+ *0.55*	+ *0.55*	+ *0.64*							
	32	+ *0.52*													
	39		+ *0.46*			+ *0.53*	+ *0.64*	+ *0.64*							
Means		0.59		0.55		0.70	0.59	0.62	0.64	0.62		0.48	0.67		0.50

* No.—Guinea pig number
† S—Skin test reaction
‡ L—Leukocytic sensitivity (CCI)
CCI italicized are in the range of specific tuberculin sensitivity.

TABLE 2

MEAN COMPARATIVE CYTOTOXIC INDICES FOR THE 1:100, 1:1,000, AND 1:10,000 DILUTIONS OF OLD TUBERCULIN

Week after initiation of first therapy		2nd	5th	9th	12th	14th	18th	23rd	28th	36th	40th
Week after initiation of second therapy										5th	9th
Group	Dilution of OT	Mean CCI	Mean CCI	Mean CCI	Mean CCI	Mean CCI	Mean CCI	Mean CCI	Mean CCI	Mean CCI	Mean CCI
Uninfected animals treated and untreated	10^{-2}	1.01	1.00	0.99	1.00	1.00	1.02	1.00	1.01	1.00	1.00
	10^{-3}	0.98	1.01	0.99	1.00	1.00	1.01	1.00	1.00	1.00	1.00
	10^{-4}	1.01	1.00	1.00	1.01	1.00	1.00	0.99	1.00	1.00	1.01
Infected animals treated once	10^{-2}	0.58	0.60	0.71	0.88	0.85	0.74	0.81	0.78	0.85	0.51
	10^{-3}	0.69	0.76	1.00	0.84	0.98	0.90	0.85	0.81	0.79	0.87
	10^{-4}	0.76	0.73	0.98	0.92	0.99	0.93	0.98	0.87	0.83	0.79
Infected animals treated twice	10^{-2}									0.98	1.00
	10^{-3}									1.02	1.00
	10^{-4}									1.00	1.02
Infected animals untreated	10^{-2}	0.59	0.55	0.70	0.59	0.62	0.64	0.62	0.48	0.67	0.50
	10^{-3}	0.58	0.67	0.80	0.66	0.79	0.71	0.79	0.58	0.70	0.59
	10^{-4}	0.85	0.78	0.75	0.79	0.66	0.82	0.83	0.55	0.59	0.56

the present study by the fact that the variation in the comparative cytotoxic indices of the untreated infected animals tested with the 1:100 dilution of tuberculin was much less (standard deviation 0.0779) than it was for the nontuberculous animals. It is not possible at present to tell what factor or combination of factors is responsible for these variations in the indices of normal animals. It has been determined, however, that these variations are greater if the test substance is used in a concentration that is slightly toxic to the normal leukocyte.

Certain of the treated animals gave indices that appeared to be inconsistent with the over-all results. Some of these were subject to interpretation; for example, animal *No. 15* when tested in the fortieth week gave negative results with both the skin and the leukocyte tuberculin sensitivity tests. The fact that the animal was moribund at the time the tests were performed could account for the lack of sensitivity. Inconsistencies in the response of the leukocytes to different dilutions of OT are not so readily interpreted. For example, the cells of animal *No. 23*, in the twenty-third week, were negative to 1:100, positive to 1:1,000, negative to 1:10,000 dilutions of tuberculin. In the twenty-eighth week, the cells were negative to 1:100 and 1:1,000 but positive to 1:10,000; and in the thirty-sixth week, the cells were again sensitive only to the 1:1,000 dilution of tuberculin. Some of the results which appear inconsistent with the general picture are artefacts resulting from the calculation of the comparative cytotoxic index. In border-line sensitivity, when the effect of the OT on the cells is relatively slight, the nonspecific effect upon the cells of the control animal may result in the indices

being above the range of specific sensitivity. This emphasizes the necessity of choosing a dilution of the test substance that will cause no inhibition of the leukocytes of the control animals.

In spite of the variations obtained in this study, the findings appear to be quite definite; the leukocytes of nontuberculous animals will give indices significantly higher than those of infected animals, and infected animals that are treated until the disease becomes inactive will possess leukocytes with either lessened or no sensitivity to OT.

Discussion

From the results of this study it appears that the tuberculin sensitivity of the leukocytes of guinea pigs infected with *M. tuberculosis* decreases or disappears entirely as the disease becomes inactive. If the disease remains inactive the leukocytes continue to react to tuberculin in a manner similar to that exhibited by the leukocytes of nontuberculous animals. If, on the other hand, the disease again becomes active, the leukocytes regain their sensitivity to tuberculin. The skin appears to remain sensitive for a much longer period of time than the leukocytes, as demonstrated by the fact that one of the animals in this study had lost its leukocytic sensitivity by the ninth week of therapy but maintained a positive skin reaction until the end of the study, thirty weeks later. It appears however, that the degree of skin sensitivity may decrease in time, as indicated by the one animal (*No. 15*) which had a negative tuberculin skin reaction to the two higher dilutions shortly after the termination of the first course of therapy, but remained sensitive to the lowest dilution.

Although no injury to the leukocytes of the treated animals could be detected, it is not impossible that they may have suffered some injury. Other investigators (3–6) have reported that different types of cells show different degrees of injury in tissue cultures when exposed to tuberculin. In this study, the method which was employed tested primarily the sensitivity of the polymorphonuclear leukocytes since their greater capacity of migration allows them to travel farther than the other blood leukocytes even under adverse conditions.

It would be of interest to determine whether the difference noted between the skin and leukocyte sensitivity extends to other types of cells, such as fibroblasts, lymphocytes, and spleen macrophages.

If the sensitivity of the leukocytes of human beings follows a pattern similar to that of infected guinea pigs, it would explain some of the results obtained in the previous study (1). The condition of some of the minimal and moderately advanced cases in human subjects, as well as many of the inactive cases, might well correspond to the condition of the experimental animals in this study during the last week of therapy, that is, the disease was inactive or it was approaching a state of inactivity. Because of this the leukocytes were no longer sensitive although the skin tests were still strongly positive. Should this prove to be the case, lack of leukocytic sensitivity might be used as an indication of progress toward the inactive state and of a continuation of this state after the patient's discharge from the sanatorium.

Summary

A group of guinea pigs experimentally infected with *Mycobacterium tuberculosis* H37Rv that were skin and leukocyte sensitive were treated with streptomycin and isoniazid. It was found that, as the disease became inactive, the leukocytic sensitivity disappeared although the skin sensitivity persisted.

Sumario

El Efecto de la Quimioterapia sobre la Sensibilidad Leucocítica a la Tuberculina en los Cobayos Infectados Experimentalmente con el Mycobacterium Tuberculosis H37Rv

Un grupo de cobayos infectados experimentalmente con el *Mycobacterium tuberculosis* H37Rv y que eran cuti- y leucocito-sensibles fué tratado con estreptomicina e isoniacida. Se observó que, a medida que la enfermedad se volvía inactiva, desaparecía la sensibiliadd leucocítica, aunque persistía la cutánea.

Resume

L'effet de la chimiotherapie sur la sensibilité leucocytaire à la tuberculine chez le cobaye infecté expérimentalement avec le Mycobacterium tuberculosis H37Rv

Un groupe de cobayes a été expérimentalement infecté avec le *Mycobacterium tuberculosis* H37Rv. Ces animaux, qui présentaient une sensibilité cutanée et leucocytaire, furent traités avec la streptomycine et l'isoniazide. A mesure que l'affection se stabilisait, on observait la disparition de la sensibilité leucocytaire alors que la sensibilité cutanée persistait.

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MYCOBACTERIA FROM COLD-BLOODED ANIMALS[1]

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Introduction

Recent discoveries of new species of acid-fast bacilli pathogenic for man (1–4) and animals (5) have stimulated interest in the search for other pathogenic mycobacteria. The association of atypical acid-fast bacteria with infections normally attributed to *Mycobacterium tuberculosis* has led to the realization that tuberculous infections may not necessarily be due to the latter organism (6–18). Before Koch (19) announced the discovery of the tubercle bacillus, attempts were made to transfer the disease from humans to cold-blooded animals.

Observations

Tuberculosis in Fishes

Villemain (20) was probably the first to mention that tuberculosis existed in fishes and in some invertebrates. The first detailed descriptions of the disease in fishes, however, were given by Dubard and his associates (21–24) who isolated an acid-fast organism named *Bacillus piscium* (*Mycobacterium piscium*) from some carp. The organism was not pathogenic for man or warm-blooded animals, but various species of cold-blooded animals were susceptible.

The disease was not common among free-living cold-blooded species. Bertarelli and Bocchia (25) found only a single case in more than 100 specimens examined. A tuberculous liver was found in a green lizard (*Lacerta viridis*), but acid-fast bacilli were not demonstrated.

Johnstone (26) and Alexander (27) found acid-fast bacilli in smears made from a portion of a cod (*Gadus callarias*). The skin was discolored and suggestive of lupus in humans, but it was not possible to isolate the organism. Guinea pigs inoculated with tissue emulsions developed localized reactions but no tuberculosis.

Tuberculosis-like disease was described in a halibut (*Hippoglossus hippoglossus*) (28–30) and other marine (31, 32) temperate and tropical freshwater fishes (32–40).

Fishes are highly susceptible under aquarium conditions (41), and epidemics have been so severe that every fish upon reaching a certain age died of the disease (34, 35).

Upon examination, Winsor (32) found that 306 of 11,500 salt-water fishes were tuberculous. Only 4 of 10,000 strictly fresh-water fish were diseased. Forty species were represented; 36 were marine, and 4 were fresh-water fish. Besse

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(39, 40) reported tuberculosis in 29 different species of fishes representing 25 genera.

In general, diseased animals show lack of appetite, change in color, progressive emaciation, sluggish movements, deformities, abdominal enlargement, necrotic and caseating lesions, external white patches, and exophthalmia (22, 24, 34, 35 37, 39–44).

Although tubercle bacilli were demonstrated in many species of fishes, only the following were properly classified; *M. piscium* (21), *Mycobacterium marinum* (31), *Mycobacterium platypoecilus* (36), and *Mycobacterium anabanti* (39).

A survey of the literature revealed that tuberculosis has been described in 10 orders, 34 families, 85 genera, and 123 species of fishes.

Tuberculosis in Amphibia

Spontaneous tuberculosis in frogs was first described by Küster (45) and by Rupprecht (46), who found tubercles on the liver surface of infected animals. Acid-fast bacilli were found in smears of the lungs, but no tubercles were found.

Weber and Taute (47) reported that the slime of the aquarium housing their frogs contained tremendous numbers of acid-fast bacilli. These organisms could invade the animals' bodies but were not pathogenic. This invasive ability was demonstrated by adding virulent tubercle bacilli to the frog aquaria. The ground internal organs of these frogs produced tuberculosis in guinea pigs, while organs from frogs containing the saprophytic acid-fast flora did not infect guinea pigs.

Küster (45) examined 200 frogs from various parts of Germany and upper Italy and found characteristic lesions and acid-fast bacilli in the livers of 3 of them. The organisms grew at 30°C., but 37°C. was fatal. This strain was pathogenic for all cold-blooded animals tested.

None of the frogs examined by Bertarelli and Bocchia (25) were found to be tuberculous.

The acid-fast strain isolated from a frog by Lichtenstein (48) could be adapted to grow at 37°C. It was pathogenic for salamanders and frogs, but mice and guinea pigs were resistant. Inguinal swelling occurred only when massive doses were inoculated.

Griffith (30) and Lucas (49) isolated acid-fast bacilli from the paradoxical frog (*Pseudis paradoxa*) which was pathogenic for frogs and toads but not for guinea pigs. The organism was serologically distinct from *M. piscium*, *Mycobacterium cheloni* (61), *M. marinum* (31), Wilson's terrapin strain, cayman and snake strains (30, 49, 58, 60).

Tuberculosis in Reptiles

About twenty years after Villemain (20) mentioned that tuberculosis occurred in reptiles, the disease was described in a snake (*Tropidonotus natrix* var. *murorum*) which died in a zoological garden.

The disease was also described in the boa constrictor (50, 54, 55), several species of python—*Python moluris* (54), *Python reticulatus* (57), *Python sebae* (58, 59), and an unidentified species (56); the vivacious snake (*Tarbophis fallax*)

(30, 49 58, 60), puff adder (*Bitis arietans*), red rattlesnake (*Crotalus exsul*), checkered keelback (*Natrix piscator*), Aesculapian snake (*Coluber longissimus*), kingsnake (*Lampropeltis getulum holbrooki*) (58, 59), *Coluber catenifer* (55), and *Thamnophis sirtalis* (127). Lederer (41) also mentioned that he found acid-fast bacilli in snakes and vipers, but gave no bacteriologic details.

Two investigators (54, 56) noted that the snakes had been fed tuberculous birds, and one of the investigators (56) believed the source of infection was avian. It is interesting to note that the strains isolated by Griffith (59) and Lucas (49) resembled avian tubercle bacilli in the slimy characteristics of the cultures and the production of joint disease when inoculated into rabbits.

Tuberculosis in turtles and tortoises was described in *Trionyx gangeticus* (58, 59), *Trionyx triungis* (40), and *Chelone corticata* (61).

Friedmann (62–65) described the disease in 2 specimens of *Chelone corticata* which died in the Berlin aquarium. All stages of tubercle formation and typical giant cells were found. From one of the animals, an acid-fast organism was isolated which grew from 22° to 37°C. At the latter temperature the organism grew at the same rate as human tubercle bacilli, and the cultures were so similar as to be indistinguishable from them. The organism was highly pathogenic for cold-blooded animals, but reactions of warm-blooded animals varied with the species and size of inoculating dose. Since the two turtles were never in contact with each other but were cared for by a keeper who had a prominent tuberculosis, and as the organism grew at 37°C., Friedmann started a series of investigations to determine whether the organism could serve for immunization and treatment of tuberculosis (66–69).

Other reptilia in which the disease has been described are the green lizard (*Lacerta viridis*) (25), the Mexican black iguana (*Ctenosaura multispinnis*), and the rough-eyed cayman (*Caiman sclerops*) (58, 59).

Inoculation of Tubercle Bacilli from Warm-blooded Animals into Cold-blooded Animals

The association of tuberculous materials with the diseased carp (21) raised several questions. Were the carp actually infected with human tubercle bacilli? Were the organisms isolated from these fishes identical to *Mycobacterium tuberculosis* or had a transformation occurred? Could warm-blooded animals be infected with bacilli from cold-blooded animals and could cold-blooded animals be infected with bacilli from warm-blooded animals? Could the bacilli from cold-blooded animals be used for immunization and treatment of tuberculosis in man and animals?

Although *M. tuberculosis* was not described until 1882 by Koch (19) as the cause of tuberculosis, unsuccessful attempts were made by Verga and Biffi in 1868 (70) to produce the disease in frogs by inoculating them with tuberculous materials from humans.

After isolation of *M. tuberculosis*, these studies were paralleled by a number of investigators who used pure cultures, tuberculous sputum, ground tuberculous organs, or dead bacilli.

Experimental Infection of Fish with Tubercle Bacilli from Warm-blooded Animals

Conflicting results were obtained in attempts to infect fish by feeding or inoculating pure cultures or tuberculous materials. Morey (71) succeeded in producing tuberculosis in the carp, but Combemale (72) was unable to infect carp when sputum was fed or inoculated. Organisms avirulent for guinea pigs were found at the site of inoculation.

Other investigators were not in agreement with Combemale. Bataillon and Terre (73) believed that the inoculated organisms could multiply in the liver of the fishes although the virulence for guinea pigs was lost after one month. Others (74, 75) agreed that the fish do not develop disease but, contrary to Bataillon and Terre, found that the tissues of inoculated fish were virulent for guinea pigs several weeks later.

Bertarelli and Bocchia (25) produced lesions in the goldfish (*Carassius aruatus*) with human, bovine, or avian tubercle bacilli, but these did not have the characteristics of tubercles. Masses of bacilli were found in the testes, ovaries, kidneys, and liver. Since the organisms were found at sites distant from the point of inoculation, they evidently were able to invade the tissues and multiply. Up to eight months later, bacilli isolated from these fish retained their virulence for guinea pigs.

Since tubercle bacilli could survive in the body of some fishes, it was recommended that refuse of milk centrifuges be boiled before being discarded to prevent infection of fishes (76, 77) since they could become carriers of human disease (78).

Calmette (79) wrote an excellent review on experimental infection of fishes with mammalian tubercle bacilli.

Experimental Infection of Amphibia with Tubercle Bacilli from Warm-blooded Animals

Numerous attempts to infect amphibia with tuberculous materials were unsuccessful (70, 80, 81, 90, 103). Although microscopic examination failed to reveal acid-fast bacilli, the tissues of the inoculated animals produced tuberculosis when injected into guinea pigs (80).

In the majority of instances tubercles were produced by the inoculation of tuberculous materials from warm-blooded animals into frogs (30, 71, 82–90). However, although the inoculated bacilli were alive eight days to three years later (47, 84, 89–95), some investigators believed a decrease of virulence had occurred (22, 23, 60, 73, 85, 91–100). Guinea pigs inoculated with tissue emulsions obtained from these amphibia lived longer than those infected directly with human tuberculous materials.

Sion (101) and Weber and Taute (47) did not believe that any change in virulence had occurred. Tissue reactions could be obtained in frogs with living or dead tubercle bacilli, timothy grass, or grass bacillus II. The reactions were believed to be those of a foreign body.

Since Weber and Taute (104) showed that frogs were normally invaded by acid-fast bacilli from their environment, they considered investigations with these

animals as invalid since there was no guarantee that the acid-fast bacilli recovered from these animals were the same as those inoculated.

Experimental Infection of Reptilia with Tubercle Bacilli from Warm-blooded Animals

Tuberculosis which was associated with inoculation or the feeding of tuberculous birds has been described in snakes (54, 56, 71, 103, 105–108), lizards (22, 23, 87, 97, 108, 109), and turtles (86, 87, 109).

A number of species were infected experimentally (71, 103, 108), but Bertarelli (105) was able to infect *Varanus varius* only by inoculation with tuberculous sputum. Pure cultures of avian or human bacilli were unsatisfactory. Enormous doses were often required to produce infection (22, 96, 97).

Some investigators modified experimental conditions by maintaining animals at elevated temperatures (103) or by keeping the cultures at room temperature before inoculating the snakes, *T. natrix*, *Coluber aesculapians*, *Zamenis viridiflavis* (106). Human tubercle bacilli were able to survive in turtles and lizards three to twelve months without loss of virulence (86, 87, 108).

Experimental Infection of Animals with Tubercle Bacilli Isolated from Fishes

The carp bacillus (*M. piscium*) was pathogenic for many species of fishes, amphibia, and reptiles, but not for warm-blooded animals (24, 73). Pathogenicity for guinea pigs or rabbits—but not for the chicken, pigeon, or calf (110)—could be obtained by serial passage (22) or by acclimatizing the organisms to grow at elevated temperatures. Guinea pigs inoculated with these organisms died thirty-eight to sixty-three days after inoculation with typical tuberculous lesions. Organisms grown at room temperature were without effect (110) even when massive doses were employed (111).

Von Betegh (112) obtained localized lesions with the carp bacillus in several species of marine fishes, but only the eel (*Anguilla vulgaris*) was infected (113–115).

Feeding *M. piscium* to goldfish (116) did not produce the disease; but inoculation into the eye, muscles, or peritoneum produced a generalized tuberculosis. A saprophytic acid-fast organism isolated from the aquarium was not pathogenic for the goldfish on inoculation. Frogs were susceptible and developed tubercles when inoculated with *M. piscium* (100, 117).

The acid-fast bacillus isolated by Aronson from different species of fishes was not pathogenic for the goldfish (31). The tadpole was susceptible (118). Baker and Hagan's platyfish strain (36) produced a low-grade infection in goldfish, but feeding was without effect. Platyfish were infected whether inoculated or fed. *M. anabanti* was pathogenic for sunfishes on inoculation.

Acid-fast bacilli isolated from frogs were usually pathogenic for cold-blooded animals (30, 60) although massive doses could produce tubercles or localized swelling in some warm-blooded animals (45, 48). Guinea pig passage increased the virulence of these strains (119).

Without doubt, the greatest amount of research was carried out with the

acid-fast strain isolated by Friedmann from a turtle (61–68). The organism could grow at 37°C., and Friedmann believed it was related to human tubercle bacilli. The organism was generally not pathogenic for warm-blooded animals although it could infect the cold-blooded species. It was considered a transitional species between tubercle bacilli from cold-blooded and warm-blooded animals (120). Reactions in cold-blooded animals were considered as nonspecific or similar to reaction to a foreign body (121, 122).

Other investigators (123–126) did not consider Friedmann's organism as innocuous, since injection produced abscesses and rabbits died following intravenous inoculation.

Mycobacterium thamnopheos (127) was pathogenic for cold-blooded but not for warm-blooded animals (127, 128), but the strains isolated by Griffith from *Python sebae* (59) caused pseudotuberculosis in guinea pigs. Rabbits inoculated with this organism developed lung tubercles and knee joint involvement.

Transmutation of Acid-fast Bacilli by Animal Passage

The association of the carp with the excreta of a tuberculous patient led Bataillon, Dubard, and Terre (21) to believe that *M. piscium* was a transmutated species of *M. tuberculosis*. Since this new organism was innocuous to warm-blooded animals (73, 98), the writers attempted to show this transformation experimentally by inoculating animals with human tubercle bacilli and then tried to reverse the procedure by producing a virulent strain from nonpathogenic strains from cold-blooded animals (22–24, 96–98, 110, 129). This transformation was confirmed by Moeller (130) who, in association with the above writers, concluded that passage of human tubercle bacilli through the slow worm (*Anguis fragilis*) resulted in a transformation identical with that obtained in the carp (131).

Modification of human tubercle bacilli was claimed by passage through frogs (100, 132–134) or salamanders (135, 136), and continued passage increased pathogenicity for the cold-blooded host (85). Although cultures obtained by frog passage were no longer pathogenic for guinea pigs, they could not serve for immunization (133, 134).

Klimmer used his salamander-passaged organism for immunization of cattle (135, 136). Treated animals did not develop tuberculosis when they mingled with tuberculous cattle or when inoculated with virulent tubercle bacilli. Moeller was unable to prevent the disease in challenged animals when he used his slow-worm-passaged organism for immunization (137), but vaccinated animals did live longer than untreated controls.

In reviewing the literature, Steriopulo (138) concluded that different tubercle bacilli and pseudotubercle bacilli were varieties of the same species, and one strain could acquire the attributes of the other during experimentation.

Not all transmutation experiments were successful. Despeignes (80) was unable to modify tubercle bacilli through passage in cold-blooded animals. The inner organs of such inoculated animals produced tuberculosis when injected into guinea pigs. Other investigators failed to induce transformation when tuberculous

sputum, organs, or pure cultures were fed or inoculated into fishes, amphibia, or reptiles (74, 75, 95, 112, 113, 115, 139).

The passage of human or bovine strains of tubercle bacilli through various species of amphibia, reptiles, and invertebrates did not decrease pathogenicity or modify its characteristics (86, 95, 101, 105, 106, 140–143). Cohn (84) could not isolate acid-fast bacilli that would grow at room temperature after passage of tubercle bacilli through frogs.

The presence of a tubercle does not necessarily indicate the presence of pathogenic acid-fast bacilli since the inoculation of saprophytes has produced tuberculosis-like processes in dogs, guinea pigs, rabbits, mice, pigeons, and frogs (144, 145).

The Use of Tubercle Bacilli from Cold-blooded Animals for Treatment and Prevention of Tuberculosis

Friedmann (61–69) and Moeller (148) claimed that their organism could serve for immunization and treatment of human and animal tuberculosis. Other investigators found such treatment of little or no value (137, 146–151) or potentially dangerous (152) even though the organisms did not cause tuberculosis on injection. Inoculated animals survived for shorter (153) or longer (154) periods than controls.

The use of the Friedmann strain and other organisms from cold-blooded animals in the treatment and prevention of tuberculosis has been the subject of a great many studies. These have been reported in the *Deutsche medizinische Wochenschrift*, *Berliner medizinische Wochenschrift*, and *Muenchener medizinische Wochenschrift* from 1904 for more than twenty years.

The subject of cold-blooded tuberculosis has been reviewed by Weber and Taute (104), Klopstock (155), Calmette (79, 156), and Küster (87).

Discussion

The failure of early investigators to reach agreement on various phases of research in tubercle bacilli from cold-blooded animals was probably due to many factors: In too many instances there was no mention of controls; frogs were not pre-tested for the presence of acid-fast bacilli which, as shown by Weber and Taute (47, 104), may have come from their environment; inocula were not standardized; and animals were sacrificed as early as eight days after injection so that comparisons with animals sacrificed after longer periods, or allowed to die naturally, were not possible. The possibility of mixed cultures, as shown by Weber and Taute, may have been overlooked.

Indeed some of Friedmann's cultures were reported as being contaminated by other than acid-fast bacteria.

Weber and Taute (47, 104) noted that frogs in an aquarium could be infected with human tubercle bacilli by adding the organisms to their water. Since the frogs harbored saprophytic acid-fast bacilli in their bodies, the isolation of any acid-fast strain would depend upon conditions favorable to the type involved. Saprophytes would be isolated by incubation at room temperature and pathogens at 37°C. Thus it may not have been a question of transmutation, but of failure to recognize that 2 different strains were present at the same time.

Failure to apply statistics probably led to many erroneous conclusions, notably in the use of small numbers of experimental animals. Also, there may have been an intense interest in having a technique accepted or a driving conviction that "the" cure had been found.

The continued passage of a mixture of human tubercle bacilli and frog-harbored saprophytic strains through the frog would gradually reduce the relative numbers of *M. tuberculosis* while the saprophytic flora could continue to survive and multiply. Thus, after a number of passages, there would be relatively few cells of *M. tuberculosis* and many saprophytes so that inoculation of guinea pigs with these mixed cultures would be equivalent to injecting dosages lower than those received by animals inoculated with pure cultures of *M. tuberculosis*. Under these conditions it would be expected that the former animals would live longer.

In diseased states, similar reactions may occur as a result of infection by different organisms. Under the proper conditions, a normally nonpathogenic organism could infect a particular individual without being dangerous to others. Personal idiosyncrasies or physiologic conditions may make one individual more or less susceptible to such infection. This has been demonstrated in protozoan infections in which the presence of a nutritional deficiency has resulted in a disease of decreased severity (157). Modification of diet may create conditions favorable to intestinal parasites (158–160); and even rabbits, which are ordinarily refractive to infection with *Endamoeba histolytica*, have been infected by first altering their diet (161, 162).

The isolation of a particular organism from a patient does not necessarily mean that the organism is the cause of the disease. The patient may have been receptive or even offered a favorable environment for the growth of the organism. On the other hand, repeated isolation of an acid-fast organism from a patient showing symptoms of tuberculosis should lead the physician to consider the organism as a possible cause of the infection even though the bacillus does not resemble *M. tuberculosis*. Living organisms can adapt to unusual conditions, and acid-fast bacilli other than *M. tuberculosis* have been found repeatedly in lung diseases.

Considering the great stress laid upon human and animal tuberculosis, it was natural that other mycobacteria would be neglected. Because of their lack of pathogenicity, the rapidity of their growth, and the ease with which they may be cultivated, the acid-fast bacilli from cold-blooded animals could serve as valuable tools in metabolic studies of this group of organisms. Information gleaned with these bacilli may serve to throw light on the requirements of the more pathogenic members of the genus and perhaps help lead the way to the cultivation of those strains which have as yet not been grown in the laboratory. The present writer (163) has studied the metabolism and serology of the cold-blooded acid-fast strains and has obtained information which may be of value in the cultivation of other mycobacteria in synthetic media.

Summary

Following the discovery of a diseased carp living in a pool contaminated with the excreta of a tuberculous patient, investigations were made for the presence of

tuberculosis among several species of cold-blooded animals. Since a number of infected animals were associated with tuberculous persons or were fed tuberculous birds, an attempt was made to convert experimentally human tubercle bacilli into cold-blooded types and to utilize these "altered" strains for treatment and prevention of tuberculosis.

The success or failure of these investigations can be explained in a number of ways. There was limited use of controls, failure to appreciate the possibility of statistical variation, and failure to utilize standard strains and techniques. A low-grade immunity may have been produced in some instances, but this led some investigators to the firm conviction that "the" cure for tuberculosis had been found. Interest was justifiably directed to the question of tuberculosis in humans so that the potential value of these innocuous cold-blooded strains as tools for mycobacterial research was overlooked.

Sumario

Micobacterias Procedentes de Animales de Sangre Fría

A continuación del descubrimiento de una carpa enferma que vivía en una piscina contaminada por las deyecciones de un tuberculoso, se llevaron a cabo investigaciones relativas a la presencia de tuberculosis en varias especies de animales de sangre fría. Como varios animales infectados habían estado asociados con personas tuberculosas o habían consumido aves tuberculosas, se trató de convertir experimentalmente bacilos tuberculosos humanos en bacilos del tipo de los de sangre fría, utilizando después estas cepas "alteradas" para el tratamiento y la prevención de la tuberculosis.

El éxito o el fracaso de estas investigaciones pueden explicarse de varios modos. Fué limitado el uso de testigos, no se apreció la posibilidad de la variación estadística y no se utilizaron cepas y técnicas normalizadas. En algunos casos, puede haberse producido una inmunidad de orden inferior, pero esto condujo a algunos investigadores a la firme convicción de que se había descubierto "la" curación de la tuberculosis. El interés se encaminó justificadamente hacia los aspectos humanos de la tuberculosis, de modo que pasó inadvertido el valor potencial de estas inocuas cepas hematocrías como instrumentos para la investigación micobacteriana.

Resume

Mycobactéries d'animaux à température variable

A la suite de la découverte d'une carpe infectée dans un étang contaminé par les déjections d'un individu tuberculeux, des recherches ont été entreprises pour découvrir la présence de la tuberculose parmi plusieurs espèces d'animaux à température variable. Comme un certain nombre des animaux infectés étaient en contact avec l'infection tuberculeuse ou étaient nourris avec des oiseaux tuberculisés, on a tenté de convertir expérimentalement des bacilles tuberculeux humains en types de bacilles d'animaux à température variable et d'utiliser ces souches "modifiées" pour le traitement et la prévention de la tuberculose.

Le succès ou l'échec de ces recherches peut être expliqué de diverses manières: il y a eu limitation de l'emploi de témoins, absence d'évaluation de la variation statistique, les souches et les techniques standardisées n'ont pas été utilisées. Une immunité de degré réduit peut avoir été réalisée dans quelques cas, mais ceci a entraîné certains des investigateurs à avoir la ferme conviction que "la" cure de la tuberculose avait été découverte. Il est compréhensible que l'attention ait été dirigée vers les aspects humains de la tuberculose, mais on a négligé la valeur potentielle de ces souches inoffensives pour les recherches sur les mycobactéries.

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Case Reports

SILICOSIS OF OCHER WORKERS[1]

A Clinical and Anatomo-pathologic Study

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INTRODUCTION

In the south of France, in Provence, and more particularly in the Apt district (Department of Vaucluse) near Avignon, there exist numerous deposits of earthy ochers which give rise to an ocher industry. The ocher mines are in a clay and sand substratum in which ferric compounds are incorporated. The principal importance of this ocher lies in its coloring properties and qualities, with such varied industrial applications as the manufacture of varnish, lacquer, linoleum, et cetera. People who work with ocher or handle ocherous materials are exposed to pneumoconiotic hazards due to the fact that the substance is mainly composed of silica and also contains a large proportion of iron. The present writers have been able to observe a number of workers suffering from this type of silicosis.

History: Ocher mining in France dates back to the Second Empire and reached its peak before World War I, when Russia was the principal consumer. The industry depends mainly upon export, as France herself uses only 5 per cent of production. Since the Russian market has been lost, Germany, Scandinavia, Egypt, and Greece have become the main buyers.

The ocher industry must compete with manufacturers of synthetic dyes; in 1911, 40,000 tons were sold, but since 1945 the annual sales have not exceeded 15,000 tons.

The city of Apt is the chief center of ocher mining in France.

Chemical composition of ocher: The ocherous earth of Apt consists of cretaceous sands admixed with clay and ferric oxide. The latter gives ocher its yellow color when hydrated, red when anhydrous. The proportions of ferric oxide and clay range between 10 and 25 per cent and 75 and 90 per cent, respectively, in Apt ocher. The clay consists of iron silicates or a mixture of silicates of aluminum, potash, or soda; however, the percentage of pure silica included in such clay is variable. The composition of a typical ocher is as follows: iron 2 O3: 17 per cent; silica O2: 51 per cent; aluminum 2 O3: 23 per cent. The 50 per cent silica value of Apt earth is significant.

Ocher mining. Various methods: In this study, it is necessary to consider mining practices and the various processes used in producing powdered ocher in its final form.

In Vaucluse, there are two companies producing ocher, i.e., Compagnie des Ocres de France (C.O.F.) and Société des Ocres Françaises (S.O.F.). The ocher is mined in ores or in open quarries, depending upon the thickness of earth covering the deposits. In ores the work is most noxious. "Ocher washing" is the next process and is aimed at separating the commercial ocher from sandy residues. In this process, water is run over the ocher, takes the ocher in suspension, and is then flowed into a decantation basin where the ocher settles out. Ocher washing takes place in winter, from November to May. In spring and in the course of summer, the ocher dries in these basins and is finally stored in the form of briquettes. These briquettes are then pulverized in grinding and bolting shops. The fineness of the impalpable powder thus produced corresponds to screen N° 300, e.g., 240μ. Despite

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the application of safety devices, men who work in these shops are exposed to dust inhalation and, as a consequence, to a greater risk of silicosis.

Clinical Study

For a long time the present writers have examined periodically with roentgenography all workers from Apt who were occupied in the exploitation of ochers, e.g., an annual average of 250 examinations.

Up to the present day, only a score of really abnormal roentgenograms have been observed that can be incorporated in the standard classification of the different stages of this disease. It is believed to be of interest to present briefly some of these observations.

Case Reports

Most frequent pseudotumoral forms

These pseudotumoral forms appear to affect men of fifty years of age who have been working for more than fifteen years in the mining of ocher. Four of the known patients died.

Case 1 (CIT). The patient stopped his work at the age of fifty-one years after twenty-three years of service in the ocher factories. A chest roentgenogram taken four years before his death, caused by cachexia, showed two important oval masses in the right lung and a less well-defined one in the left lung. In the hili, there were egg-shell-like lymph nodes.

Case 2 (VER). The patient had been working for nineteen years in the mine before he finally gave up work at fifty-four years of age. The chest roentgenogram revealed two enormous balloon-shaped masses surrounded by parenchyma in the upper lobes, emphysema in the bases, and dilatation of the right heart (figure 1). He died at the age of fifty-seven.

Case 3 (RIC). The patient had been employed in the manipulation of ocher for sixteen years. He abandoned his activities at the age of fifty and died five years later. The roentgenographic images were less dense and less well defined than in the preceding observations.

Case 4 (THAL). The patient died in the writers' department in December, 1955, at the age of sixty-three. During the last four years he had had signs of chronic bronchitis and emphysema which obliged him to abandon the work he had carried on for eighteen years. The chest roentgenogram showed the presence of some well-defined and perfectly round tumor masses in each lung field. In the hilar areas, especially on the left, there was a clump of lymph nodes with well-defined lines and a clear center, emphysema in the bases, and enlargement of the vena cava superior.

The following 3 patients are more than sixty years of age and have been working between eighteen and twenty-one years in the mines. If respiratory insufficiency has impeded their efforts, it has certainly not reduced them to inactivity.

Case 5 (CAP). In the patient's left lung, the roentgenogram showed two spheroid masses in the left parahilar area and homogeneous condensation to the right.

Case 6 (IMB). A recent chest roentgenogram revealed two tumor masses at the apices, and small and large nodules were seen on their periphery. Pulmonary emphysema was also present.

Case 7 (COL). This patient had never actually worked in the ocher field but, since 1936, had run an estate on ocher land. In September, 1953, he was admitted, as an urgent case, for spontaneous pneumothorax. There were significant tumor masses in the upper lobes.

Case 8 (GUID). Chest roentgenography in this case revealed small nodules scattered throughout the lungs and an excavating tumor mass to the right.

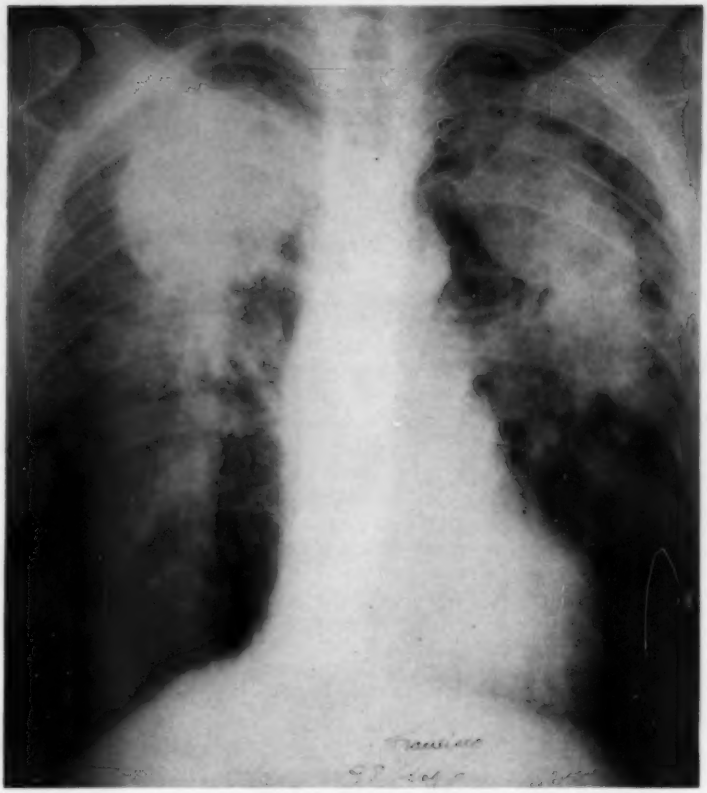

FIG. 1, *Case 2*. Chest roentgenogram showing large masses (balloon-shaped masses, fire balloon forms, "formes en Montgolfières") in the upper lobes of lungs and emphysema at the lung bases.

Nodular and confluent forms

The following cases are those of workers who have each been exposed for eighteen years to ocher dust.

Case 9 (VAC). The patient was sixty-one years of age. The roentgenogram revealed fine spots of nodules on the lung fields, tending toward confluence. Some lymph node enlargements were present.

Case 10 (COR). The 71-year-old patient appeared to be in perfect physical condition. The chest roentgenogram revealed disseminated nodulation with confluence in certain zones, egg-shell-like lymph node enlargements, and emphysema.

Case 10 bis. (VOL). The patient was fifty-six years of age. The roentgenogram of the lungs showed small nodules disseminated throughout the upper lobes. In the hilum there were egg-shell-like lymph node enlargements.

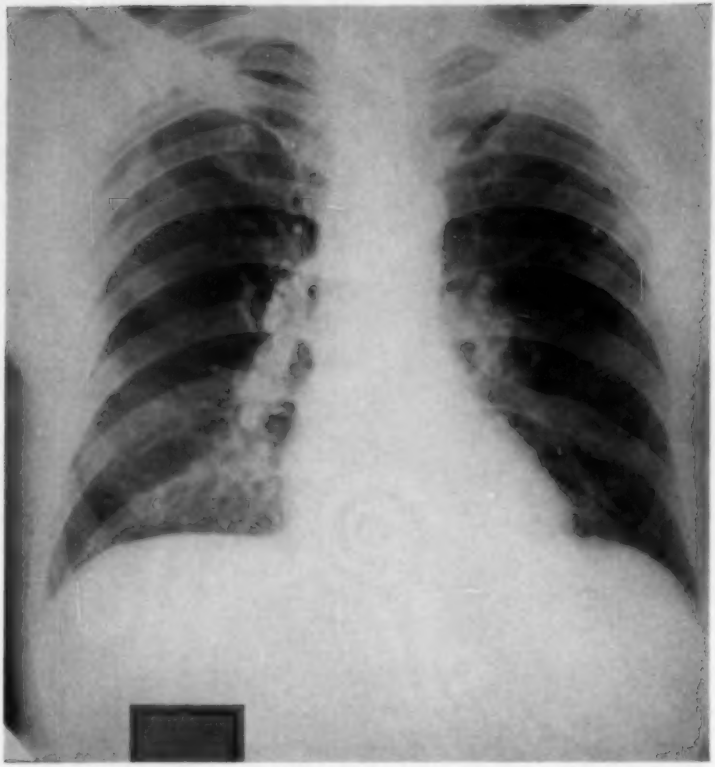

FIG. 2, *Case* 11. Roentgenogram of lungs showing normal parenchyma. There are several hilar lymph nodes (egg-shell-like or mother-of-pearl-like calcified nodes).

Trabecular forms

Case 11 (CAS). The patient was forty-six years of age and had been working with ocher for five years. At the level of the hilum, multiple lymph node enlargements formed a "bunch of grapes" on the right and a clump on the left (figure 2).

Case 12 (SCA). The patient had been working with ocher for nearly thirty years. The chest roentgenogram showed increased bronchovascular markings and a few enlarged lymph nodes.

A search for *M. tuberculosis* has been carried out systematically in all 12 cases. Cultures, gastric washings, and animal inoculation have always proved negative. On the other hand, 2 cases of ocher silicosis complicated by tuberculosis have been observed.

Complicated forms

Case 13 (ROS). The patient was a 51-year-old man. He had previously had a trabecular image in the lung fields and subsequently developed characteristic pulmonary tuberculosis.

Case 14 (VEL). In this patient, a cavitary tuberculosis with many tubercle bacilli in the sputum developed among tumor masses which had formed during the course of four years.

The silicosis passed through three stages. The subject, who was born in 1900, had worked on ocher for only seven years.

Roentgenographic Findings

Ocher silicosis appears to present certain unusual roentgenographic findings which deserve mention.

The tumor masses are different from those usually observed in coal miners. They are localized in the upper lobe and show balloon or "en Montgolfière"[2] forms, rising toward the apices and attracting the trachea in their direction.

These masses are spherical or oval and most of them are well defined and surrounded by a layer of parenchyma, even on the sides of the mediastinum. The "butterfly" forms seen in coal miners are not encountered in the men who have been working with ocher. The emphysema in the basis is widely spread, as in any silicosis.

In the nodular forms of ocher silicosis, the small and the large nodules, which show a general tendency toward confluence, may sometimes be found on the same film. Sometimes they appear to have a little density. The present writers have never seen "snowstorm"-like pictures or the pseudogranulous aspect present in certain other types of silicosis.

Among the patients suffering from ocher silicosis, there are nearly always bilateral adenopathies, which have the aspect of "egg shells" as Grayson named them in the United States in 1936. Lined with a calcified border, these lymph node enlargements show a clear central part which also evokes the image of a mother-of-pearl button; they are arranged in clumps, sometimes quite far from the hilum. Such enlarged lymph nodes presumably represent pseudotumoral forms with a feeble evolutive potential. This was confirmed by the present observations. In actuality, the present writers have not found any noncalcified and voluminous adenopathies which would indicate evolutive silicosis.

Pathologic-chemical and Cristallographic Analysis

Pathology: The examination of the lungs of the patient THAL, who died, as mentioned above, from a pseudotumoral silicosis, resulted in the following observations. (It is believed that this represents the first autopsy of a patient with ocher silicosis.)

Macroscopic examination (Dr. Roche and Dr. Chemin): In each lung, on a level with the upper lobe, a rounded tumefaction was present. Puncture of one of the tumors with a needle showed a rather thick liquid, the color of lentil juice and containing small solid deposits. A section made at the level of the spheroid tumor showed that the latter, the size of a Chinese orange, presented a 2 mm. thick envelope surrounding a brown concrete muddy liquid in the central part. The reddish-brown parenchyma had lost all elasticity around the border and had a cheeselike consistency. The pleura had undergone an enormous fibrous thickening. Numerous lymph nodes were scattered about the hilum and varied in size from a pea to an almond. From the trachea and the altered bronchi a brownish yellow mucus flowed freely. There was no evidence of macroscopic lesions. The heart was enlarged and heavily infiltrated with fat.

Bacteriologic findings: The necrotic material was negative for tubercle bacilli on culture and on guinea pig inoculation.

Histologic findings (Professor Picard, Laboratory of Histology, Faculty of Medicine, University of Marseille): It was very easy to make a distinction between the common lesions, some of which depended on cardiac insufficiency, and specific lesions which can be included in the common description (1).

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[2] "Fire balloon" or "tennis-racket" forms.

The sclerosis, first of all, showed multiple forms. They were systematic, perilobular, and in well-defined, more or less star-shaped foci. There were large areas of extensive sclerosis with a few typical fibrohyaline nodules. The pseudotumoral masses were as well defined in their borders as the small fibrohyaline nodules, and showed an identical structure. The central necrosis, which was almost total in some of these masses, had no connection with their dimensions, for some of the small nodules were excavated in the same way.

The resulting necrotic mass was granular and cell-free and in places showed prints of numerous and voluminous crystals, which were removed with the solvent during the fixation. The edges of such constituted cavities had the appearance of atheromatous necrobiosis. An examination under polarized light showed that the crystals found were "cholesterides" (Professor Picard). In other respects, the smears were constituted of amorphous materials and very fine birefringent microcrystals. This central aseptic excavation of the large blocks of sclerosis resembled undoubtedly the description given by Gernez-Rieux and associates (2) concerning coal workers. The vicinity of those sclerotic areas very often showed vascular lesions, including either a granulomatous layer of the adventitia or an obliterating endarteritis.

Another fundamental aspect of the lesion was shown by histiocytic and granulomatous areas where the parenchyma's lattice could sometimes be seen. The histiocytes were gathered either in the alveolar cavities or in the thickened and finally dislocated septa. These lesions appeared to be either isolated or at the level of the peribronchi, or again around the nodules and the large sclerotic areas. The mineral elements were unusually distributed: the fibrohyaline masses, the blocks of sclerosis with or without sclerosis very seldom showed calcified deposits; but above all there was a large amount of fine brown dust consisting of sharp-edged microcrystals.

The same microcrystalline elements were found in the granulomatous areas often phagocyted by histiocytes; some granules with a positive iron test were located in the same place. The cytoplasm of the frequently very large histiocytes gave a diffuse reaction of unequal intensity with the iron test, but they showed a number of grains or rounded lumps similar to pigments of hemosiderin. With the lowest power of magnification, the iron test sometimes gave pictures with a "cocarde" form[3] around the nodules similar to the differential distribution which is found in the dusts of antracosilicosis (Frühling). The sclerosed and silicohyaline formations, the necrotic masses, gave a negative result to the iron test.

In short, from the histologic point of view, it appears that slightly polymorphous lesions represent all of the usual modalities of silicosis, and, more especially, nodular formation, extensive fibrosis, and necrosis; in addition, they show ferric deposits, the nature of which will be discussed.

The voluminous hilar lymph nodes show reticular and fibrous modifications that are common in pneumoconiosis: microcrystalline deposits, but only very small quantities of ferric pigments.

Chemical analysis (by Professor Dumazert, Department of Pharmacy, University of Marseille):

Analysis of the excised pulmonary wall:

Water content	77.2%
Dry weight at 110° C.	22.8 gm.
Total nitrogen (Kjeldahl)	2.60 gm.
Ashes (content)	4.10 gm.
Iron (Fe)	0.80 gm.
Silica (Si O2)	1.10 gm.
Calcium	0.14 gm.
Aluminum	in traces

[3] Concentric bands of the same colors with different tonalities.

After incineration of a pulmonary fragment, the resulting ashes washed out with hypochloric acid left an important silica residue; the iron tests made with these ashes gave a highly positive reaction.

Cristallographic findings (by G. Perinet, Laboratory of X-Ray, National Center of Scientific Industrial and Naval Researches at Marseille): Using the X-ray diffraction tests, an attempt was made to search the form of the silica and of the iron detected by chemical analysis on one hand and, on the other hand, to make a comparative study of these elements with a sample from the ocherous earth with which the patient worked. Without going into technical details, it should be pointed out that two types of Debye-Scherrer spectra have been used in order to obtain an oscillating diagram, on one hand using the plate form of the lung sample, and a powder spectrum, on the other hand.

The two spectra show the lines of quartz and goethite (a variety alpha of iron hydrate Fe 2 O3, H2 O) and the arch of kaolinite.

It was necessary to establish the comparison with these spectra and those obtained from the Apt ocher. In the latter case, the diagram also showed three intense spectra: quartz, goethite, and kaolinite.

General Comment

This general study leads to a few reflections. Only a few people suffer from ocher silicosis in the Apt district; the few cases are certainly predisposed. This disease develops during the course of several years and results in a pseudotumoral form with a slow evolutive potential if compared with the very accentuated roentgenographic signs (tumor masses with a balloon shape associated with some characteristic lymph nodes with an egg-shell-like and mother-of-pearl-like buttons).

The specific characters of the ocher tumor masses should be emphasized. From both the roentgenographic and the clinical points of view, the tumor masses correspond with the last stages of the evolution of the disease. This stage is usually found in workers exposed to dust during a long period (mixing or grinding), as Policard and Roche (3) insisted, and in workers generally between fifty and seventy years of age. These masses appear to have only a remote effect on the respiratory and cardiovascular functions.

The roentgenographic aspect of these formations is homogeneous. However, as mentioned above, the post-mortem puncture of one of the tumors produced several milliliters of a granulous, brownish, and aseptic liquid. This indicates that these condensations which are apparently solid can, in fact, mask losses of substance. Moreover, a softening of such masses is always possible. This latter finding supports the statements made by previous writers (Gernez-Rieux, Lille) and Kilpatrick (4) about the aseptic cavitation of the anthracosilicosis of coal workers.

The autopsy described can be compared with those gathered by Symanski (Sarrebruck) (5) concerning the iron workers (Salzgitter mines). The latter writer found enlarged lungs colored with ferruginous dust; on the histologic analysis he found, besides silicotic lesions, abundant quantities of ferric pigments. The study of Even (6) on the pneumoconiosis of the iron workers of French mines of Lorraine also establishes sidero-silicosis.

The X-ray diffraction tests have added an important contribution to the study in identifying, in the lung tissues of the miner examined at autopsy, the same crystallized quartz as that detected in the ocher dust which was gathered on the actual work fields. Besides quartz, a spectrum analysis showed two other elements: goethite (alpha variety of iron hydrate Fe 2 O3 H2O) and kaolinite. Ocher penumoconiosis is therefore a silicosiderosis resembling, by the presence of clay, the porcelain silicosis studied by de Leobardy of Limoges and, above all, the colored earth pneumoconiosis studied by German writers (7–9).

In connection with the presence of goethite, the problem of ferric deposits should be discussed (Picard). According to recent research, it seems impossible to distinguish histologically whether a ferric pigment is of exogenous or endogenous origin (10). In the present case, these pigments are too numerous to be of an endogenous and hemosiderinic origin. Their topographic distribution is very unusual; they gather in the interior of the histiocytes conglomerated around the fibrous nodules, whereas in all of the sclerotic areas the iron test is negative. This leads to speculation whether the dust constituents could be separated during the elaboration of the lesion or later on. This latter interpretation can in fact be put forward.

If the iron oxide does not form a chemical combination with the other constituents, it is therefore bound to the clay through indetermined forces. For, it cannot be separated *in vitro* until after the attack of the clay by aqua regia; and, in the liquid, the iron blue test is then intensely positive, revealing the dissolved iron. *In vivo*, it presumably would produce an analogous reaction. The chemical action of the plasmatic or cellular medium upon the mineral is rather obscure, but it is now generally admitted that a small quantity of silica can be dissolved in the organism. In the same way, a slow attack of the clay can be envisaged and an incorporation in the solution of the free iron. At this point, the extreme affinity of the histiocytes for iron would take place; the iron would be then trapped in a dissolved state and incorporated into a lipoprotein complex elaborated by the cells (10). The iron can even determine this histiocytic reaction in a pure state and without sclerosis, "miliare des soudeurs à l'arc" (11).

It is reasonable to believe, therefore, that, if the ferric deposits of an exogenous origin remain *in situ* for an indefinite period, it is because of their quantity, which results in the mmobilization of the histiocytes.

Summary

The writers have made a complete survey of ocher silicosis in the Apt region (Department of Vaucluse, France). They have furnished a report of the autopsy performed upon an ocher worker who had been mining ocher for a period of eighteen years.

Ocher pneumoconiosis gives rise to a sidero-silicosis and, as the silica content is highly significant, the pseudo-tumoral forms are encountered quite often (balloon tumors or Montgolfière tumors, with egg-shell- or mother-of-pearl-like ganglions). These masses, which are roentgenographically homogeneous, can show a softening process in the center. This finding is very similar to that which occurs in necrotic fibrosis of anthracosilicosis. The liquid produced, as well as the tissues, was free of tubercle bacilli.

By using the X-ray diffraction tests, it has been possible to make a comparison between a sample of the lung and a specimen of ocherous earth, which has permitted the writers to individualize—for one as well as the other—a common spectrum, including in its lines the essential constituents of ocher dust: quartz, goethite (alpha variety of iron hydrate Fe2 O3, H2O), and kaolinite.

Sumario

La Silicosis de los Mineros de Ocre: Estudio Clínico y Anatomopatológico

Versa este minucioso estudio sobre la silicosis por ocre observada en la región del Apt (Departamento de Vaucluse, Francia). Comprende también una reseña de la autopsia ejecutada en un sujeto que había minado ocre durante un período de dieciocho años.

La neumoconiosis ocrosa produce una siderosilicosis y, como el contenido de sílice es sumamente elevado, obsérvanse bastante a menudo las formas seudotumorales (tumores en globo o estilo Montgolfier, con gangliod parecidos a cascarón de huevo o nácar). Estas tumefacciones, radiográficamente homogéneas, tal vez revelen un proceso de reblandeci-

miento en el centro. Este hallazgo es muy semejante al observado en la fibrosis necrótica de la antracosilicosis. El líquido producido, e igualmente los tejidos, estaban exentos de bacilos tuberculosos.

Usando las pruebas de difrección de los rayos X, ha sido posible trazar una comparación entre una muestra del pulmón y un ejemplar de tierra ocrosa, que ha permitido individualizar—para una y otro—un espectro en común, que comprende en sus líneas los componentes esenciales del polvo de ocre: cuarzo, goethita (variedad alfa del hidrato de hierro, Fe2 O3,H2O) y caolinita.

Resume

La Silicose des travailleurs de l'ocre. Etude clinique et anatomo-pathologique

Les auteurs font une étude complète de la silicose de l'ocre dans la région d'Apt, près d'Avignon. Ils rapportent l'autopsie d'un ouvrier ocrier ayant travaillé 18 ans dans une mine d'ocre.

Les pneumoconioses de l'ocre donnent lieu à une sidéro-silicose et, la teneur en silice étant importante, les formes pseudo-tumorales sont souvent rencontrées (tumeurs en ballon ou en Montgolfière avec présence de ganglions en coquilles d'oeufs ou en boutons de nacre). Ces formations, radiologiquement homogènes, peuvent être le siège de ramollissement, puisque la ponction peut ramener un liquide granuleux "jus de lentille" ou "vert épinard".

Cette constatation est rapprochée de ce qui se passe dans les fibroses nécrotiques de l'anthraco-silicose. Les liquides prélevés aussi bien que les tissus, ne renfermaient pas de bacilles de Koch.

La comparaison, par diffraction aux rayons X, d'un prélèvement pulmonaire, d'une part, et d'un échantillon de la terre d'ocre, d'autre part, a permis d'invidualiser, pour l'un comme pour l'autre, un spectre commun, comprenant dans ses raies les constituants essentiels de la poussière d'ocre quartz, goethite (variété alpha de l'hydrate de fer Fe2 O3, H2O) et kaolinite.

Acknowledgment

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OBSTRUCTION OF THE SUPERIOR VENA CAVA PRESUMABLY DUE TO HISTOPLASMOSIS[1]

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INTRODUCTION

Obstruction of the superior vena cava is generally considered to be an unusual clinical entity (1, 2). Only 5 per cent of such obstructions are due to inflammation of the veins or mediastinum (3). In a series of sixty cases of mediastinitis (4), Keefer found twelve which were chronic, and in two there were no clues to etiology. Only one case of this syndrome caused by histoplasmosis has been found in the literature (5), despite the fact that mediastinal lymphadenopathy is frequently discovered on the chest roentgenograms of patients who have had histoplasmosis. Because of the increased interest and incidence of histoplasmosis (6) and because venous obstruction in the mediastinum is rarely mentioned when the disease is discussed, it was thought best to report 2 additional cases.

CASE REPORTS

Case 1. N.G., a 23-year-old poultryman, was admitted to the Mary Fletcher Hospital in January, 1956, for shortness of breath associated with swelling of the face and neck. The dyspnea had begun when the patient was ten years old. At that time he had an illness thought to be rheumatic fever. It was interesting to note that approximately three years after this illness a brother of the patient died from a disease involving the mediastinum, which was diagnosed as Hodgkin's disease.

In November, 1955, after dressing a large number of turkeys, the patient noted swelling of the face and neck sufficient to require an increase in his collar size. His dyspnea became worse, and any coughing, straining, or effort to lie down produced a marked increase in the swelling associated with visible cyanosis. The cyanosis was limited to the upper thorax, head, and arms. He had an occasional dry, hacking cough unassociated with any hemoptysis, chills, fever, weight loss, or symptoms referable to other parts of the body.

Family history was noncontributory except for the death of the brother mentioned above.

The patient had lived all of his life on a Vermont farm. Physical examination at the time of admission revealed a temperature of 37°C. Pulse was 88 per minute and regular; respirations, 18 per minute; and weight, 130 pounds. The patient was small, muscular, and alert, with no evidence of dyspnea at rest. His face appeared cyanotic, and there was visible swelling which also involved the neck, arms, and upper thorax, to such a degree that the lower half of the body appeared somewhat underdeveloped in contrast. No abnormality of any sort was noted below the thoracic cage. Arterial pulsations were strong and equal in all extremities. There was no evidence of clubbing or lymphadenopathy.

The retinal veins and the sub-lingual venous plexus were distinctly prominent. The veins in the neck and arms were visibly distended and failed to empty regardless of position. The neck appeared full, measuring 16½ in. in circumference, and no mass could be palpated. The trachea was in the midline. A number of abnormally prominent veins were visible over the anterior chest (figure 1). These veins did not pulsate. The thoracic cage was symmetrical with equal motion bilaterally. The lungs were clear throughout on examination, except for the presence of rather high-pitched, and somewhat harsh breath sounds heard only over the right hilar region anteriorly. A harsh, mid-systolic cardiac murmur could be heard in this same localized region and radiated out through the right lung field but was strikingly

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FIG. 1. Illustration showing venous distention in arms and neck with development of collateral venous drainage in anterior chest wall. Note swelling of the face and eyes.

absent in the left chest. The heart was otherwise normal on examination, all sounds being clearly heard. There was no thrill associated with the localized mid-systolic murmur. The blood pressure in both arms was 132 systolic, 60 diastolic in mm. of mercury. The remainder of a complete physical examination failed to reveal any abnormalities.

Blood examination revealed a hemoglobin content of 15.0 gm. per 100 ml., with 5,130,000 erythrocytes per cu. mm. and 7,100 leukocytes per cu. mm. A differential revealed neutrophils, 62 per cent; stab forms, 3 per cent; lymphocytes, 31 per cent; monocytes, 3 per cent; and eosinophils, one per cent. The hematocrit was 47 per cent. The erythrocytes sedimenta-

tion rate was corrected to 29 mm. per hour. A serologic test for syphilis (Mazzini) was negative. The urinalysis showed a specific gravity of 1.026 with no abnormalities. A tuberculin skin test using second-strength PPD was read as doubtful. The histoplasmin skin reaction was strongly positive, producing a vesicle in the center of a 3 cm. area of redness and induration.

The venous pressure was 390 mm. of water. The total serum proteins were 8 gm., with 3.9 gm. of albumin and 4.1 gm. of globulin per 100 ml. The prothrombin activity was more than 50 per cent. The agglutination test for brucella was negative.

Roentgenograms of the chest revealed some widening of the mediastinum with prominence of the right hilar shadows in a manner strongly suggestive of calcified nodes (figure 2) and the lateral film confirmed this impression. There was no evidence of a parenchymal lesion in either lung field and no evidence of pleuritic involvement. The cardiac contour was entirely within normal limits.

The patient was afebrile while in the hospital. He was comfortable as long as he could lie in a semi-upright position, but rapidly became cyanotic and dyspneic when he attempted to lie flat.

A diagnosis of obstruction of the superior vena cava was made. Intravenous dye, failing to enter the superior vena cava at any point, clearly confirmed this diagnosis (figure 3). The dye passed through collateral channels which included the vertebral, azygos, hemiazygos, and internal mammary channels and various channels of veins in the anterior thoracic cage. It should be specifically stated that the dye that entered the right azygos system failed to empty into the superior vena cava in the usual manner but continued downward, entering the inferior vena cava at a lower level. This complete obstruction of the superior vena cava appeared related to the numerous calcified shadows that extended upward from the subcarinal area along the right side of the mediastinum, resulting in a narrowing of the right bronchial tree as demonstrated by the planigrams. The calcified lymph nodes also presumably compressed the right pulmonary arteries sufficiently to produce the systolic murmur described above. Several other smaller calcified shadows were noted on the planigrams in the mediastinum, and numerous calcified shadows were demonstrated in the spleen (figure 4). There was no evidence of a parenchymal lesion within either lung field.

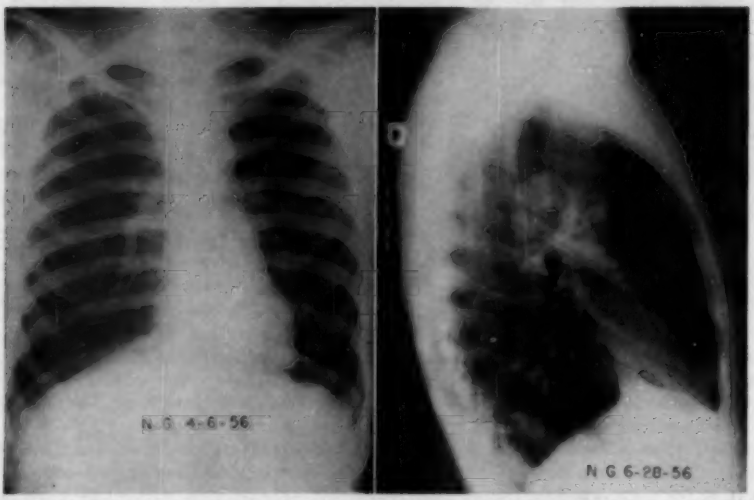

FIG. 2. Showing abnormal shadow in right hilar region suggesting some calcification. Mediastinum was slightly widened. Lung fields were relatively clear.

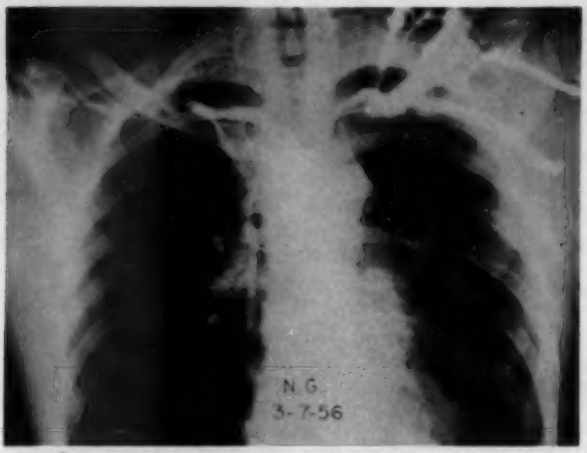

FIG. 3. Diodrast® introduced in left arm is shown passing through right innominate vein and azygos in other collateral channels. The superior vena cava was demonstrated to be completely obstructed.

The patient was discharged from the hospital but returned one month later with essentially the same findings. A right scalene node biopsy was done, and grossly abnormal nodes were removed. Hard nodules thought to be lymph nodes could be palpated deeper in the mediastinum than would be possible to reach safely through the supraclavicular incision. The pathologic report was chronic lymphadenitis, probably secondary to obstruction. *Histoplasma capsulatum* could not be demonstrated despite efforts to do so with special staining techniques and careful cultures.

Repeated skin tests showed exactly the same findings and the coccidioidin skin reaction was also negative. Complement-fixation tests performed (by Dr. Michael Furcolow) were reported as positive in dilution of 1:4.

A diagnosis of chronic calcific mediastinal lymphadenitis due to *Histoplasma capsulatum* was made. The process was believed to be active and encroaching upon the right bronchial tree and pulmonary artery. After careful consideration it was decided that surgical intervention should not be attempted unless absolutely necessary. When the patient was last seen, approximately eighteen months after the initial visit, the signs and symptoms were essentially unchanged, despite a prolonged course of anticoagulants.

Case 2. The second patient was a 30-year-old housewife first seen in September, 1956, because of a swollen vein on her chest. Past history revealed that the patient had suffered as a child from swelling and bluish discoloration of the left arm following exertion, accompanied by congestion and throbbing in the head. At the age of eight she began having attacks of "pleurisy" with cough, fever, and chest pain, lasting up to one week. At twenty years of age she had a severe cold with the first of many ensuing attacks of wheezing. Pleurisy was quite frequent until the age of twenty-four. Three years later during her second pregnancy, at the age of twenty-seven, she noticed a swollen vein on the anterior chest and wheezing on exertion. Twice she coughed up blood, at one time as much as four ounces. Chest roentgenograms taken then were similar to those described in this paper. After an uneventful termination of this pregnancy, the wheezing and dyspnea progressed and the number of dilated veins on the chest and abdominal wall and in the neck increased. Symptoms and signs progressed without interruption until she was first seen at this hospital. She had lost thirty pounds in weight in the previous six months despite a good appetite.

FIG. 4 (Top). Roentgenogram showing calcified shadows in spleen and in lower left mediastinum. (Bottom). Planigrams showing compression of right bronchus by calcified lymph nodes. Note calcified nodes lower on the left.

There was palpitation but no fever, sweats, or dysphagia. The patient's mother had asthma. The patient had lived for six months before her complaint began with an uncle who was ill and who later died of "tuberculosis." Except for one winter in Florida, this woman had always lived in Vermont where her childhood had been spent on a farm.

Physical examination revealed a temperature of 37° C. Pulse was 100 per minute and regular; respirations, 20 per minute; and weight, 130 pounds. She was a well-developed, well-nourished woman with flushing of the face. Examination of the head, eyes, nose,

mouth and ears was negative, but the neck showed several dilated veins. On the chest there was a large, tortuous varicose vein extending from above the right breast diagonally downward to the left costal margin at the mid-clavicular line. This vein was shown to fill from above. Several other large superficial veins on the anterior chest were connected with this one, as were a few veins on the lateral chest wall (figure 5). The lungs were resonant on percussion, and the breath sounds were normal with no rales or rubs. The heart was not enlarged and the sounds were of good quality and regular with a soft, aortic, systolic murmur heard in the erect position. The blood pressure was 128 systolic, 86 diastolic in mm. of mercury. The liver extended 4 cm. below the right costal margin, with a sharp, firm, slightly tender edge. The spleen was not palpable. The veins in the arms were distended but no edema was found. All of the extremities showed normal pulses and reflexes without clubbing or edema.

The blood examination revealed a hemoglobin content of 12.6 gm. The leukocyte count was 8,450 per cu. mm. with neutrophils, 68 per cent; lymphocytes, 27 per cent; monocytes, 4 per cent; and eosinophils, one per cent. The hematocrit was 38 per cent. The corrected erythrocyte sedimentation rate was 14 mm. per hour. A urinalysis showed a specific gravity of 1.024 but was otherwise negative. A serologic test for syphilis was negative. An agglutination test for brucella was positive in a dilution of 1:20. Two histoplasmosis complement-fixation tests were done (through the kindness of Dr. Michael Furcolow) and reported as positive in a 1:8 dilution. A bone marrow culture was negative for *H. capsulatum* after six

FIG. 5. Showing engorgement of collateral veins on anterior chest wall.

weeks of incubation. Three tuberculin skin tests were done; all were negative. A histoplasmin skin reaction was positive on three occasions. Skin reactions for coccidioidin and blastomycin were negative. The venous pressure in the right antecubital vein was 224 mm. of water on one occasion and 235 mm. on another. An electrocardiogram was normal.

A roentgenogram of the chest showed slight prominence of the right atrial region. There was moderate widening of the supra-aortic shadow and questionable prominence of the vasculature of both lungs, more marked on the left (figure 6). Phlebograms which were done

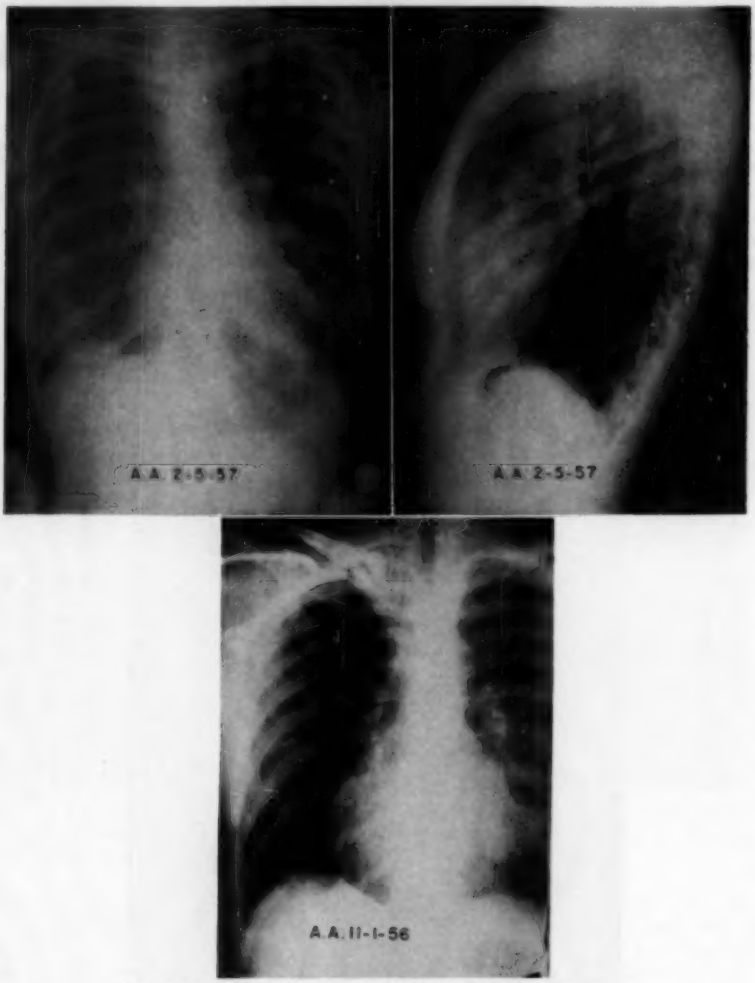

FIG. 6 (Top). Densities in left lower lung are clearly seen.

FIG. 7 (Bottom). Urokon® injected into right antecubital vein meets obstruction at junction of subclavian and innominate veins and regurgitates into neck veins.

(figure 7) showed the dye entering the antecubital vein and flowing upward, reaching an obstruction near the junction of the subclavian and innominate veins. No dye could be seen in the superior vena cava but there was slight filling of a few collateral veins in the supraclavicular area. Planigrams of the upper thorax showed a widening of the cardiac shadow and a few calcified nodes in the peribronchial and paratracheal regions.

The patient was seen again six months later. At this time she stated that the swelling and congestion were worse. There was frank edema of the head and neck and engorgement of some arm veins. She was placed on Dicumerol® and discharged for home treatment.

Discussion

The clinical manifestations of histoplasmosis vary from a mild illness, usually of the upper respiratory tract, to an acute fulminating type that is usually fatal. Evidence is accumulating that more and more of the mild cases have been misdiagnosed (7–10). The usual symptoms in their order of frequency as reported in one study (11) were cough, expectoration, chest pain, fever, fatigue, hemoptysis, and wheezing. Lymphadenopathy, splenomegaly, a moderate anemia, and leukopenia are usually noted (6). The lungs are the most frequent site of disease, which often is ushered in by pneumonia that is resistant to all forms of treatment (12–15). There may be involvement of other systems and the symptoms will be referable to the system involved. Chronic mediastinitis has been reported once in the literature (5).

Histoplasma capsulatum can withstand freezing for more than a year and can tolerate heat up to 45°C. for half an hour. These findings help to explain the frequency with which the organism is found in the soil and in the dust of long-unoccupied buildings (16, 13, 10). The organism has also be recovered from the air (13).

The diagnosis of histoplasmosis has been made either at necropsy in the acute fulminating cases or by the discovery of pulmonary calcification which ultimately led to the correct diagnosis. The usual onset is an acute upper respiratory infection (5, 9–11, 13, 17–19). A history of farm employment with exposure to dust from the excreta of chickens, turkeys, or pigeons is highly suggestive. Although some writers believe that the diagnosis may be sufficiently substantiated without demonstration of *Histoplasma capsulatum* (9, 10, 18), it is generally believed that definite establishment of the diagnosis must rest on recovery of the organism from the patient (12, 15, 20, 21). The results of the individual skin test depend upon several factors. A negative reaction may be found in acute fulminating histoplasmosis (6). Patients with old calcified lesions are said frequently to have negative reactions. Positive skin reactions must be assumed to indicate probable activity. Cross-sensitivity with coccidioidomycosis and blastomycosis serves to confuse this picture (15, 22). Skin tests should be made for all three fungi in order to determine which gives the most marked reaction.

The same factors that influence the skin test also affect the complement-fixation test. The complement-fixation titer is said to be at its height in about three months, after which it may slowly fall (21).

Although the above criteria may all be very suggestive, the fact remains, as mentioned previously, that the secure establishment of the diagnosis of histoplasmosis can be made only by isolating the organisms. As the literature is examined, it will be noted that only a small percentage of the reported cases was found to fulfill all of these requirements, and this applies also to the 2 cases here reported.

The signs and symptoms of superior vena caval obstruction depend upon the location of the lesion, the rapidity of its extension, and the rate of development of collateral anastomoses. Slow progressive involvement may produce very little difficulty (23). The location of the obstruction will determine the routes of collateral anastomoses.

Aside from the primary disease, the symptoms are produced by the increased venous pressure. Patients show edema and swelling of the face, neck, and arms. Cyanosis of the face, lips, and ears is prominent. Dyspnea is not unusual. Headache, epistaxis, somnolence, and convulsions are reported. Cough and dysphagia are usually present in the course of the disease.

When suggested by the above signs and symptoms, the site of the obstruction can usually be located by the use of intravenous dye (24).

The 2 cases presented have many features in common. The patients must have developed the infection at an early age. This observation, stressed in the literature (1, 4–8, 17), obliges physicians in all areas, endemic or not, to remember histoplasmosis when diagnosing cases of upper respiratory infection. The reason for aggravation in the early twenties is not apparent from the history, although the first patient may well have incurred a secondary infection when he dressed the turkeys. Although the skin reactions were positive for histoplasmosis, they were negative for coccidioidomycosis and blastomycosis and, for practical purposes, to both strengths of PPD. The first case had a complement-fixation of 1:4 on the first test and a negative reaction six months later. The second case had a serologic agglutination of 1:8. The organism was not recovered from either patient, although a search was made.

Summary

Two cases of superior vena caval obstruction presumed to be due to histoplasmosis have been reported, only one such case having been reported previously.

Sumario

Oclusión de la Vena Cava Superior Debida Presuntamente a Histoplasmosis

Se presentan 2 casos de oclusión de la vena cava superior, debida presuntamente a histoplasmosis, no habiéndose comunicado antes más que un caso de este género.

Resume

Obstruction de la veine cave supérieure probablement dûe à l'histoplasmose

Deux cas d'obstruction de la veine cave supérieure présumés dûs à l'histoplasmose sont rapportés; il n'y avait eu qu'un seul cas rapporté antérieurement.

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PYRAZINAMIDE-INDUCED ACUTE YELLOW ATROPHY OF THE LIVER[1]

Case Report and Post-Mortem Findings

THEODORE N. SPENGOS AND ROD CUIZON

(Received for publication November 5, 1957)

Introduction

The use of pyrazinamide in combination with other antituberculous drugs is not new (1–4) and the effectiveness of this two-drug regimen has been emphasized. In this hospital, pyrazinamide-isoniazid has been used as preoperative and postoperative chemotherapy in patients with tubercle bacilli resistant *in vitro* to isoniazid, para-aminosalicylic acid (PAS), and streptomycin. It has also been used in nonsurgical cases of far advanced pulmonary tuberculosis in which drug-resistant tubercle bacilli are in predominance and progression of the disease has occurred (6).

The toxicity of pyrazinamide is well known (2). In this hospital the pyrazinamide dosage used was 1 to 1.5 gm. along with 300 mg. of isoniazid each day. Although the dosage employed (30 mg. per kg. of body weight daily) is considered relatively low, clinical jaundice and one fatality with fulminating hepatic failure occurred (2, 5). It is the purpose of the present paper to report the occurrence of pyrazinamide-induced acute yellow atrophy of the liver and acute pancreatitis in one of the patients treated with pyrazinamide-isoniazid on this low-dosage regimen.

Case Report

F.L.B., a 65-year-old male, had been diagnosed as having moderately advanced pulmonary tuberculosis since 1953 and had been treated with isoniazid, PAS, and streptomycin intermittently for a total of twelve months prior to his admission to the Western Oklahoma Tuberculosis Sanatorium on March 17, 1956. On admission to the hospital he complained of weakness, cough, and spells of shortness of breath. He weighed 100 pounds. His sputum was positive for tubercle bacilli. A review of chest films taken in 1953, 1954, and 1956 revealed many calcifications in the upper lung fields and a moderately dense infiltrate involving the upper two-thirds of the left lung with apparent progression of disease. The patient was started on streptomycin therapy, 1 gm. daily for thirty days and twice weekly thereafter, along with isoniazid in a daily dosage of 5 mg. per kg. of body weight. This regimen was continued until December, 1956, with roentgenographic improvement; but the sputum continued to contain tubercle bacilli. Drug-susceptibility tests for isoniazid, PAS, and streptomycin in March and September, 1956, showed "partial resistance" to isoniazid and to streptomycin. By June, 1956, a large cavity at the left apex was definitely shown. The patient's chief difficulty was recurrent asthmatic attacks. By November, 1956, he developed dermatitis extending over his nose, lips, scalp, neck, and upper extremities. This hypersensitivity of the skin was attributed to streptomycin, but an attempt to put him on PAS failed because he developed upper gastrointestinal symptoms and his dermatitis became worse. Isoniazid and streptomycin were omitted for a few weeks in December and January but, despite treatment with antihistamines and local application of various ointments, there was no improvement of his skin lesions. Consequently, prednisone was given, 10 mg. three times daily for three days, and the dose was reduced to 2.5 mg. daily from February 6, 1957, until June 20, 1957. The results were excellent, with clearing of the dermatitis and remarkable respiratory relief. While on prednisone the patient had no asthmatic attacks and rarely used bronchodilators.

[1] From the Western Oklahoma Tuberculosis Sanatorium, Clinton, Oklahoma.

In March, 1957, a postero-anterior film of the chest revealed a spreading of the tuberculosis in the left upper lung and an increase in the size of the cavity. Drug-susceptibility studies of the patient's tubercle bacilli again revealed "partial resistance" to isoniazid and to streptomycin. Because of this finding and the intolerance to PAS and the allergy to streptomycin, pyrazinamide was selected as a companion drug for isoniazid for continuation of the treatment. The pyrazinamide administration was started on March 30, 1957, in a daily dosage of 1.5 gm. along with 300 mg. of isoniazid. The patient had been taking the latter drug continuously since January 1, 1957. In April, the patient also received 10 mg. of prednisone daily, and in May and June from 5 to 2.5 mg. daily (1,155 mg. total). Prednisone was discontinued on June 20, 1957. A chest film on June 18, 1957, showed improvement in the left lung under this regimen, and at his insistence the patient was granted a leave of absence.

The cephalin flocculation and icterus index prior to, and two weeks following, initiation of pyrazinamide-isoniazid therapy were normal. These tests were not repeated frequently because the patient appeared to tolerate the drug well and no clinical evidence of liver embarrassment could be detected. On July 6, while at home, he started complaining to his family of feeling sick to his stomach and became nauseated, and had no appetite. The next day he started vomiting everything ingested, and on the third day the family noticed that he had become yellow. He returned to the hospital in a stuporous condition and with a deep icteric discoloration of his skin. The following day massive ecchymotic areas were evident all over his body surface. He developed ascites and peripheral edema and went into a coma and died on July 15, 1957.

Post-mortem Examination

The gross findings at autopsy revealed the lungs to be adherent to the pleura at the apical areas, and multiple calcifications were felt at both apices. Cross sections of both lungs revealed the above-mentioned calcified nodules and a large cavity at the left upper lung field measuring 4 by 7 cm. in diameter, and containing caseous material. A smaller cavity was found at the right apex. The abdominal cavity contained dark yellow fluid. The liver was small, weighing 514 gm., and was moderately firm and rubbery. The capsule was finely wrinkled, glistening, and gray. On section, the cut surface of the liver had a

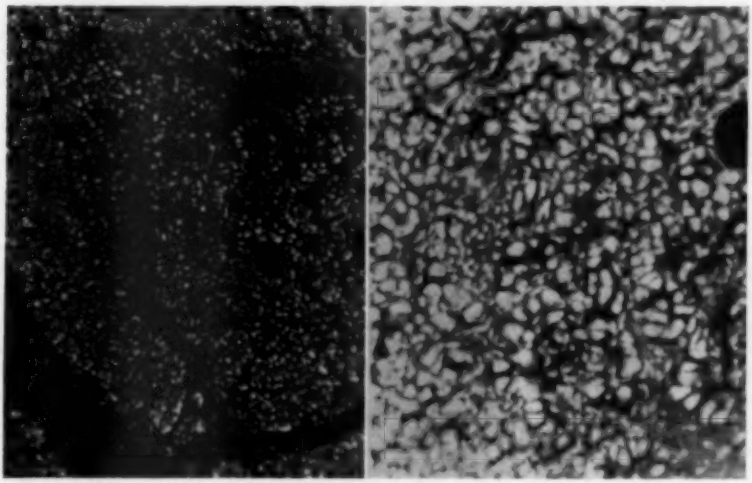

FIG. 1. Photomicrographs illustrating virtually destroyed hepatic parenchyma. (× 60)

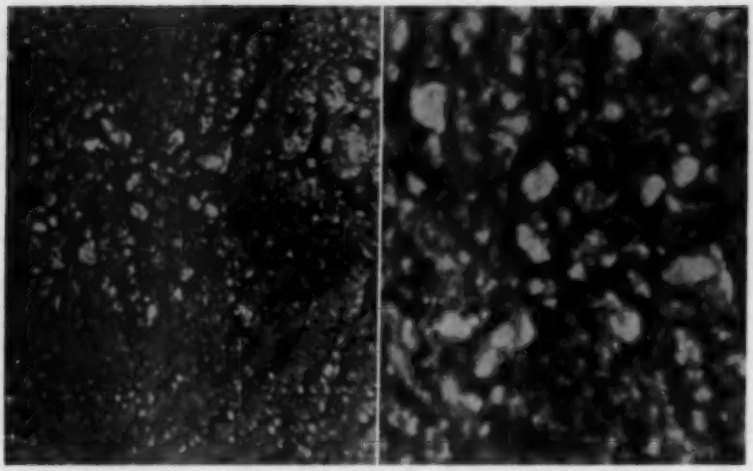

FIG. 2. Photomicrographs illustrating areas of destroyed pancreatic parenchyma and inflammatory cell infiltration. (× 200)

slightly gray to red-brown color. The liver and pancreas were of interest in their microscopic appearance.

The other organs did not present any microscopic or macroscopic findings of significance.

Liver: Sections showed almost complete necrosis of the liver cells. Some portal areas were noted which appeared to be intact. Here and there were islands of more or less intact liver cells. On the periphery some congestion was present, and marked pigmentation was noted throughout the section. From the sections examined, the liver appeared virtually destroyed (figure 1).

Pancreas: A considerable amount of inflammatory cell infiltration was noted. Much of this was polymorphonuclear leukocytes. In places the pancreatic parenchymal cells were destroyed. Much of this inflammation also extended into the pancreatic fat (figure 2). The pathologic diagnosis was: acute yellow atrophy of the liver and acute pancreatitis.

DISCUSSION

The course of events in this patient was not unprecedented because fatalities induced by pyrazinamide have been reported (1–6). Nevertheless, this case of acute yellow atrophy of the liver is reported because it was produced by what is considered a moderate dose. Pyrazinamide, along with isoniazid, was employed in this case because of intolerance to PAS and allergy to streptomycin, with severe dermatitis. The skin lesions were successfully treated with prednisone. Roentgenographic improvement was observed in nine weeks following administration of the pyrazinamide-isoniazid, but there was no disappearance of tubercle bacilli from the sputum. It may be suggested at this point that perhaps prednisone was a contributing factor to the patient's symptomatic and roentgenographic improvement. The cephalin flocculation and icterus index determinations obtained early in the course of pyrazinamide-isoniazid therapy did not give notice of the oncoming storm. Unfortunately, the patient did not offer any complaint, and consequently the administration of the drug was continued. In addition, this patient was not an alcoholic and had no previous history of jaundice. He had had no blood transfusions, and there was no case of infectious hepatitis in the hospital at that time. When he returned to

the hospital he was severely jaundiced and presented signs of impending hepatic coma. The administration of pyrazinamide was immediately stopped and he was treated vigorously with vitamin K and intravenous glucose. He died on the ninth day of the onset of symptoms.

The patient had received a total of 150 gm. of pyrazinamide in one hundred days. The dosage used in this case is considered relatively low, but hepatitis even on this low dosage has been reported (5). It is the writers' belief from experience with a group of patients who have been or are now being treated with pyrazinamide-isoniazid that there is an unpredictable individual susceptibility to pyrazinamide and that acute yellow atrophy of the liver may occur.

The autopsy findings clearly demonstrate the catastrophic changes in the liver parenchyma. Acute pancreatitis was also found by the pathologist, but it seems that it was terminal and its significance is not clear.

Summary

A case of acute yellow atrophy of the liver caused by pyrazinamide is reported. The onset of symptoms of toxicity and the fatal acute hepatic failure were very short, comprising only nine days. The pyrazinamide had been administered for one hundred days in a daily dosage of 1.5 gm. along with 300 mg. of isoniazid. It appears that liver function tests should be done more frequently during pyrazinamide administration regardless of the lack of any clinical symptoms of pyrazinamide toxicity. Despite this precaution, there may still remain the hazards of the unknown individual susceptibility to pyrazinamide.

Sumario

Atrofia Amarilla Aguda del Hígado, Inducida por la Piracinamida

Preséntase un caso de atrofia amarilla aguda del hígado, provocada por la piracinamida. La iniciación de los síntomas tóxicos y de insuficiencia hepática aguda fué muy breve (9 días) después de la medicación. La piracinamida, 1.5 gm., fué administrada durante 100 días junto con 300 mg. de isoniacida. El efecto tóxico sobre el hígado resultó letal. Parece que deben ejecutarse más frecuentemente pruebas de la función hepática, independientemente de la falta de todo síntoma de intoxicación por piracinamida. A pesar de esta precaución, quizás haya que confrontar todavía los riesgos de la desconocida susceptibilidad individual a la droga.

Resume

Atrophie jaune aiguë du foie causée par la pyrazinamide

Observation d'un cas d'atrophie jaune aiguë du foie causée par la pyrazinamide. L'intervalle entre le début des symptômes toxiques et l'insuffisance hépatique aiguë fut très court (9 jours). Une dose quotidienne de 1,50 g. de pyrazinamide et de 300 mg. d'isoniazide fut administrée pendant 100 jours. L'effet toxique sur le foie a été fatal. Les auteurs estiment que les tests de la fonction hépatique devraient être faits plus souvent sans tenir compte de l'absence de symptômes cliniques de toxicité de la pyrazinamide. En dépit de cette précaution, on peut néanmoins rencontrer une sensibilité individuelle ignorée à la pyrazinamide.

Acknowledgments

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Notes

APPLICATION OF HOOKE'S LAW TO THE ELASTIC PROPERTIES OF THE LUNG

The elastic properties of the lung have been studied by means of pressure-volume relationships obtained during moments of zero air flow. The pressure change necessary to produce a certain change in volume (usually taken as 100 or 1,000 cc.) has been termed *elastance*, and the volume change in liters produced by a one centimeter of water change in pressure has been termed *compliance*. These terms are based on concepts drawn from engineering. It is the purpose of this paper to examine these basic concepts and to see how accurately they may be carried over into the field of pulmonary physiology.

If an external force is applied to a body, an equal and opposing force is set up within the body. This internal force is called a stress. If the numerical value of the total stress is divided by the cross-sectional area at right angles to the direction of the stress, the unit stress is obtained. Thus, if a pulling force of 20 pounds is applied to a steel rod having a uniform cross-sectional area of 2 square inches, the unit stress will be 10 pounds per square inch. If the cross-sectional area is 2 square inches in one part of the rod and 5 square inches in another, the unit stress will be 10 pounds per square inch in the smaller section and 4 pounds per square inch in the larger (figure 1).

It is relatively easy to visualize the unit stress in a simple steel bar subjected to tension. If the body has a more complex shape, the problem becomes more difficult. Consider a hollow cylinder containing fluid under pressure. This pressure will act in all directions perpendicular to the surface of the cylinder, as illustrated by the arrows in figure 2. The stress, however, is developed within the walls, and can be visualized by passing a plane through the cylinder, dividing it in half. The arrows in figure 3 represent the unit stresses.

The deformation produced in a body by an external force is called the strain. The total amount of deformation divided by the initial size of the object in the dimension of deformation is the unit strain. Thus, if a bar 10 inches long is stretched one inch, the unit strain will be $\frac{1}{10}$. This represents the amount that any unit of the bar is stretched. Thus, each inch is stretched $\frac{1}{10}$ inch, and each centimeter $\frac{1}{10}$ centimeter, et cetera.

An elastic body is one which, when deformed, will regain its original size and shape when the deforming force is removed. This will hold true up to a value of unit stress called the elastic limit. If this is exceeded the body will be permanently deformed. A steel bar is an example of an elastic body, and if such a bar is subjected to a constantly increasing tension and the unit stress is then plotted against the unit strain, a diagram such as figure 4 will be obtained. The straight part of the curve obeys the equation: unit stress/unit strain = K. This relationship is known as Hooke's Law; the value of K is a constant for any given material and is known as the modulus of elasticity. Because of a similarity in terms and methods, static pressure-volume diagrams of the lung might be supposed to be examples of Hooke's Law, and the measurements of compliance or elastance might be assumed to give information about the distensibility of elastic tissue itself. Such conclusions, however, are not justified without reservations.

Two facts should be emphasized. First, Hooke's Law refers to unit stress and unit strain, and hence the constant K which represents the numerical expression of the elastic properties cannot be calculated as the quotient of total applied force and total deformation. Second, since unit stress and unit strain are used to calculate K, the value of K is independent of the dimensions of the particular object being tested. Thus, the modulus of

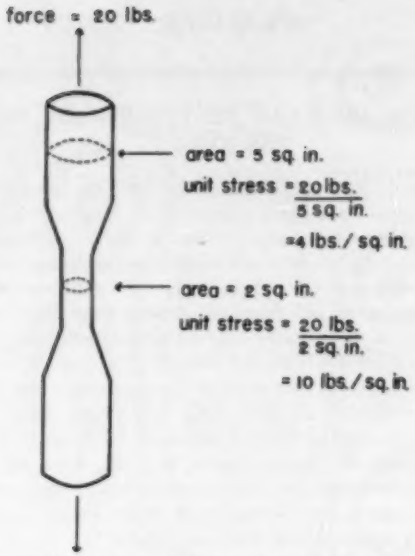

FIG. 1. Unit stress developed in a bar subjected to tension

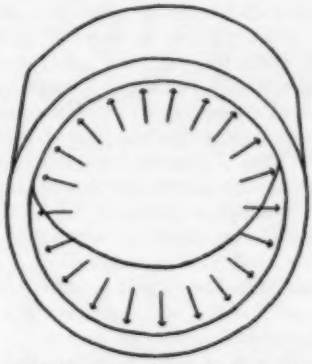

FIG. 2. Pressure acting within a cylinder

elasticity is influenced only by the physical properties of the material, and not by the structure and dimensions of the object. Modulus of elasticity is a property of a material, not of a body composed of that material.

This is not true for the value of compliance as used in pulmonary physiology, where total volume change is used in the calculation. For example, if the same amount of force is applied to two bars of the same material and cross-sectional area, one twice as long as the other, each unit of material will stretch the same amount, the longer bar stretching twice as much as the shorter. If the "compliance" were calculated by dividing the total stretch by the applied force, the longer bar would be found to be twice as "compliant" as the shorter, although they were composed of the same material.

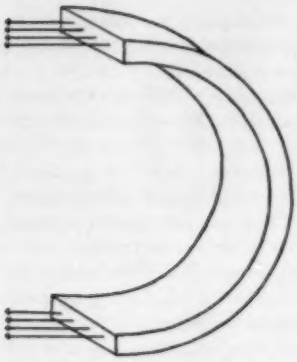

FIG. 3. Unit stresses developed as a result of the pressure illustrated in figure 2

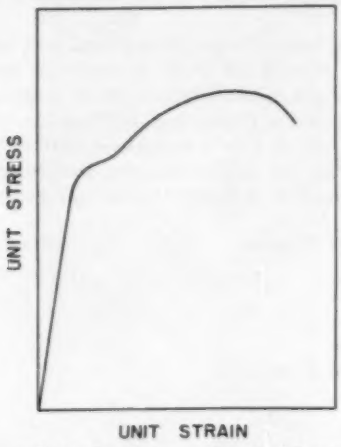

FIG. 4. Unit stress—unit strain diagram of a steel bar subjected to tension

In addition to the over-all size, the exact design must also be known in order to obtain information about the structural material itself. Referring back to the example of the hollow cylinder (figure 2), it can be shown that the unit stress in the wall can be computed from the formula $s = PD/2t$, where s is the unit stress, P the pressure, D the diameter, and t the wall thickness. Consider three such cylinders of the same shape and diameter. Cylinders A and B are made of the same material, but B has one-half the wall thickness of A. Cylinder C has the same wall thickness as A, but is made of a material which has half the modulus of elasticity. Now let all three cylinders be subjected to the same pressure. Referring to the formula, B will develop twice the unit stress of A, and will be deformed twice as much because the material is the same. Cylinder C, having the same dimensions as A, will develop the same unit stress but, since the material has a modulus of elasticity half that of A it will be deformed twice as much as A, just as was cylinder B. Thus, unless the exact shape and dimensions were known, it would be impossible to tell whether the differences in total amount of deformation were due to differences in actual structural material or to differences in size and dimension.

That size must be taken into account when dealing with the lung has been demonstrated by measurements of compliance in the cat, in which clamping off one main stem bronchus has been noted to reduce the compliance by one half.[1] It has also recently been shown that in adult human beings the compliance varies with the size of the lung.[2] Other factors such as the presence of fibrous tissue, surface tension, and distributional effects may also play a role in altering compliance.

It is obvious from the simple example of the cylinders that such a measurement as vital capacity or total capacity would be entirely inadequate for calculations of unit stress and unit strain of the lung. Thus, any information obtained from static pressure-volume relationships will be influenced by the size and structure of the lung as well as the material of which it is composed. Bayliss and Robertson[3] recognized this when they stated that elastance is a property of a system and involves its geometry. A lung with twice the compliance of another is not necessarily composed of tissues which are twice as compliant, and a normal value for compliance does not necessarily infer normal pulmonary elastic tissue. A prime example of this is the compliance measurement in chronic pulmonary emphysema, which often has been found to be normal[4-6] in spite of the known marked alterations in the elastic tissue.

In summary: Some of the basic concepts of the resistance of materials are reviewed and their applicability to the study of the elastic properties of the lung is discussed. It is emphasized that static pressure-volume diagrams of the lung approximate only roughly the stress-strain diagrams used in engineering, and therefore should not necessarily be expected to obey Hooke's Law. It is to be emphasized further that values for compliance and elastance computed from such data do not give information concerning the material of the lung alone but are markedly influenced by size and structure.

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DEPARTMENT OF MEDICINE,
STATE UNIVERSITY OF NEW YORK
UPSTATE MEDICAL CENTER
SYRACUSE, NEW YORK

November 13, 1957

[1] NISELL, O. I., AND DUBOIS, A. B.: Am. J. Physiol., 1954, *178*, 206.
[2] FRANK, N. R., *et al.*: J. Appl. Physiol., 1956, *9*, 38.
[3] BAYLISS, L. E., AND ROBERTSON, G. W.: Quart. J. Exper. Physiol., 1939, *29*, 27.
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[5] MEAD, J., *et al.*: J. Clin. Investigation, 1955. *34*, 1005.
[6] EICH, R. H., *et al.*: Am. Rev. Tubere., 1957, *76*, 22.

SHORT-TERM THERAPY IN EXPERIMENTAL TUBERCULOSIS

Although available drugs have greatly improved the outlook for the treatment of tuberculosis, they are wanting in at least three ways: they are suppressive rather than eradicative, making long periods of treatment necessary; strains of tubercle bacilli resistant

TABLE 1

COMPARISON OF KNOWN ANTITUBERCULOUS DRUGS FED FOR TWENTY AND FOR SEVEN DAYS

Compound	Twenty Days		Seven Days	
	Dose per Day	Days of Increased Survival*	Dose per Day	Days of Increased Survival*
	mg.		mg.	
Para-aminosalicylic acid	20	10.0	40	3
			20	0
4,4′-diaminodiphenyl sulfone	5	>20†	20	lethal
			10	>5
			5	0
Isoniazid	0.1	14	0.1	13
Nicotinaldehyde thiosemicarbazone	5	>20	5	>14
	2.5	>20	2.5	>9
Nicotinamide	25	12	30	>13
			25	9
p-Acetylaminobenzaldehyde thiosemicarbazone	2.5	>20	10	20
			5	12
			2.5	5
Pyrazinamide	10	>20	20	>20
	5	8	10	7
	2.5	0	5	0
Streptomycin subcutaneously	1.0	>20	2.0	>15
	0.5	12	1.0	5
	0.25	5	0.5	4
Neomycin subcutaneously	3	24	2	11
	1	7	1	8.8

* Most figures are averages of several experiments with each drug—10 mice per group.
† > Indicates that some mice were sacrificed on the fortieth day post infection.

to the drugs emerge, making the value of treatment thereafter a question of some debate; lastly, they are frequently ineffective against chromogenic strains, a variety of acid-fast organisms that appear to be showing up more frequently. With these points in mind, it seems to the writer that laboratory workers who are seeking new antimicrobial drugs for

TABLE 2

VARYING DOSAGE SCHEDULE OF ISONIAZID

Days of Treatment	Mg. of Isoniazid Fed per Day											
	0.05	0.1	0.25	0.5	1	2	3	4	5	6	7	8
1	—*	—	—	—	—	—	—	X–2†	S‡	S	S	S
2	—	—	—	—	—	—	—	S	↓	↓	S	S
3	X–10	X–7§	X–3	X–1	X–1	X–1	S	↓	↓	↓	—	—
4	—	—	—	—	S	S	↓	↓	↓	↓	—	—
5	—	—	—	—	↓	↓	↓	↓	↓	↓	—	—
6	—	—	—	—	↓	↓	↓	↓	↓	↓	—	—
7	X–10	X–3	S	—	↓	↓	↓	↓	↓	↓	—	—

* — = Not done.

† X–2 = Two of 10 mice dead before thirty-fifth day.

‡ S = Complete survival.

§ X–7 = Seven of 10 mice dead before thirty-fifth day, et cetera.

tuberculosis should revamp their procedures, so as to uncover drugs that are not just as good but very much better than those now in use.

As a start in this direction, when screening unknown agents the writer has abandoned the traditional long-term therapy of infected mice and reduced treatment from twenty to seven days, placing the drug to be tested in the feed. Most of the drugs effective in murine tuberculosis, as will be seen in table 1, exerted a significant increase in survival with this shorter period of treatment, although larger doses were usually necessary to accomplish this increase. The mice were sacrificed forty days after infection. PAS and 4,4′-diaminodiphenyl sulfone were exceptions—their efficacy apparently depends upon long-term administration.

To make the selection of new agents even more rigorous, those that showed good activity in the seven-day test could be retested by administering them in large doses for an even shorter period of time. As a base line, we investigated the ability of isoniazid to prolong life when given at various doses for varying lengths of time. The experiments were terminated thirty-five days after infection. The control survival time for these experiments was from nineteen to twenty-one days. As will be seen in table 2, 1 mg. of isoniazid for four days, or 3 mg. for three days were sufficient to keep the mice alive until the thirty-fifth day after infection. Autopsies, however, revealed that all of the mice had lung lesions, the degree of involvement being greatest in those that received the least amount of drug. Future experiments will include the concurrent administration of two or three drugs at high levels for short periods of time.

THE MERCK INSTITUTE FOR THERAPEUTIC RESEARCH
RAHWAY, NEW JERSEY

FLORENCE K. FITZPATRICK[1]

February 6, 1958

[1] With the technical assistance of Stella Pietryk, Gail Shand, and Albert Reid.

Correspondence

"SPUTUM CONVERSION" AND THE METABOLISM OF ISONIAZID

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

I read with much interest Dr. Roger S. Mitchell's editorial on "The limitations of our knowledge about para-aminosalicylic acid." I would like to take him up on one point. It has been suggested, as he mentioned, that the superiority claimed for para-aminosalicylic acid (PAS) as a companion drug to isoniazid may be explained by the elevation in many patients of the serum concentrations of biologically active isoniazid. It is the superiority of PAS as a companion drug which I wish to question. In the view of our group and of others, the reason for this conclusion lies in the fact that, in general, outside the United Kingdom—at least until very recently—streptomycin has been used intermittently with daily PAS or isoniazid. It is almost five years since the Medical Research Council of Great Britain showed,[1, 2] in a carefully controlled trial, that daily streptomycin *plus* isoniazid was superior to intermittent streptomycin *plus* isoniazid in the prevention of drug resistance. In Edinburgh we have reason to believe (though our analyses are not yet completed) that intermittent streptomycin *plus* PAS is also inferior. In all the figures I have seen from the United States it has seemed to me that the use of intermittent streptomycin combined with one other drug could well account for the failure-rate recorded.

As a preliminary to a wider survey with my colleagues of patients from all of the Edinburgh tuberculosis hospitals, I have just completed an analysis of sputum conversion in all patients with pulmonary tuberculosis admitted under my personal care from 1952 to 1956, inclusive. These patients fulfilled the following criteria: (*1*) sputum, gastric lavage, or laryngeal swab culture positive for tubercle bacilli; (*2*) tubercle bacilli susceptible to at least two of the three main drugs. There were 240 patients and the conversion-rate at different periods of time is shown in figure 1. The date of conversion is taken as the date of the first of a continuous series of cultures negative for tubercle bacilli. Although I have not yet carried out detailed follow-up studies, except for the 1952–1953 patients, almost all have been followed up under my own supervision and I know of none who has subsequently had tubercle bacilli in the sputum, although there were one or two later postoperative deaths after conversion and some have died from other diseases.

As will be seen, four cases have been excluded from the conversion graph. Details are given in the figure. The patient who was infected with PAS-resistant bacilli was treated with PAS *plus* isoniazid in the 1952–1953 Medical Research Council trials. Conversion was attained by bilateral surgery under cover of streptomycin and oxytetracycline, a valvotomy for congenital pulmonary stenosis being carried out at the same time.

Of the 240 cases, 9 per cent were minimal; 49 per cent, moderately advanced; and 42 per cent, far advanced by the U. S. National Tuberculosis Association classification.

It will be seen that the sputum was converted in all patients by the tenth month. Admittedly in 1952–1953 almost 40 per cent were also treated by surgery, though in the later years very few were so treated. The sputum was negative for tubercle bacilli in almost all patients at the time of operation.

I have to confess to an element of luck. In 1952–1953 a number of patients received intermittent streptomycin and PAS. I was fortunate to have no failures; my colleagues

[1] Medical Research Council of Great Britain, Brit. M. J. 1953, *2*, 1005.
[2] Medical Research Council of Great Britain, Brit. M. J. 1955, *1*, 435.

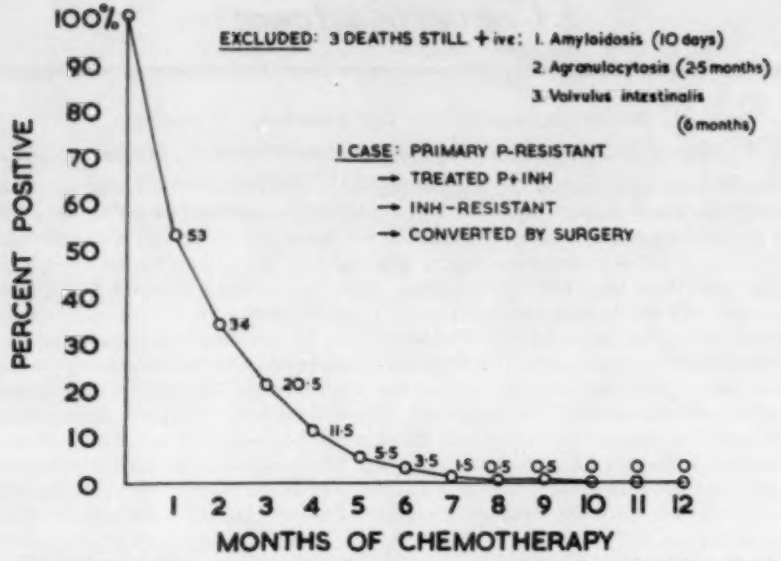

FIG. 1. Sputum "conversion" with chemotherapy, 1952-1956. Two hundred and forty patients with bacilli susceptible to at least two drugs. P—PAS; INH—Isoniazid.

have had several on this combination. The combinations actually used were as follows (when more than one combination was employed the principal pre-conversion combination is given, or when there was a doubt about which was the principal, the combination is given on which conversion occurred):

Streptomycin, 1 gm. daily, *plus* sodium PAS, 15 to 20 gm. daily: 14 per cent of cases.

Streptomycin, 1 gm. daily, *plus* isoniazid, 100 mg. twice daily: 33 per cent of cases.

Streptomycin, 1 gm., daily, *or* three times weekly, *plus* sodium PAS, 5 gm. twice daily, *and* isoniazid, 100 mg. twice daily: 32.5 per cent of cases.

Sodium PAS, 10 to 20 gm. daily, *plus* isoniazid, 100 mg. twice daily: 7.5 per cent of cases.

Streptomycin, 1 gm. twice *or* three times weekly, *plus* sodium PAS, 15 to 20 gm. daily: 12 per cent of cases.

Streptomycin, 1 gm. twice weekly, *plus* isoniazid, 100 mg. twice daily: 1 per cent (2 patients)

Para-aminosalicylic acid and isoniazid were generally given as initial treatment only in the 1952–1953 Medical Research Council trials or when the patients' bacilli were already streptomycin resistant, though we give this combination as a routine to patients after they leave the hospital or in mild cases treated as outpatients.

Despite careful studies, no drug-resistant tubercle bacilli were isolated at any time during the course of therapy or thereafter with any of the drug regimens listed. One patient was treated on isoniazid alone in the Medical Research Council trial in 1952; her bacilli became isoniazid resistant, but "conversion" was achieved on streptomycin and PAS.

It may be mentioned that of the 187 patients admitted to Edinburgh hospitals in

1953, and fulfilling the same criteria, sputum "conversion" occurred in all except one by the sixth month of treatment. The remaining patient converted in the eighth month.[3]

I should add that our sensitivity tests are most meticulous and we accept as potentially clinically significant thresholds of resistance very much lower than are general in the United States. We think that many failures on "good" chemotherapy are due to missing clinically significant pretreatment drug resistance by accepting relatively high thresholds for drug resistance, based on serum levels of the drug, instead of establishing the standards by painstaking correlation of clinical and bacteriologic data.

The obvious question regarding the data I have given is: Are they honest? I am indeed a little ashamed of the conversion results; the figures fit a curve with embarrassing precision and, as already mentioned, there has probably been an element of good fortune. There may indeed be minor errors in the analysis but I think it reasonably certain that no failure has been missed; it has certainly not been suppressed.

If individual variation in the metabolism of isoniazid were of clinical significance, streptomycin resistance might be expected in some patients given daily streptomycin with isoniazid. None occurred in this series and, prior to a full analysis, no such case can be recalled by my colleagues in this group.

DEPARTMENT OF TUBERCULOSIS AND DISEASES OF THE RESPIRATORY SYSTEM, EDINBURGH UNIVERSITY,
SOUTHFIELD HOSPITAL, EDINBURGH, SCOTLAND

JOHN CROFTON[4]

December 13, 1957

[3] Ross, J. D. et al.: Brit. M. J., 1958, *1*, 237.

[4] Dr. A. Pines kindly helped with the analysis of the 1955 patients. I am indebted to a succession of able assistants and to the meticulous nursing staff at Southfield Hospital who ensured that the drugs were taken as directed. In addition, this work owes much to the brilliant bacteriology of Dr. Sheila Stewart. Our methods are founded on the work of the Medical Research Council of Great Britain and on the group research carried out in Edinburgh with my colleagues, Drs. N. W. Horne, I. W. B. Grant, J. D. Ross, F. W. A. Turnbull and A. T. Wallace, assisted by generous financial grants from the Royal Victoria Hospital Tuberculosis Trust.

Books

DICTIONARY OF MICROBIOLOGY. By M. B. Jacobs, M. J. Gerstein, and W. G. Walter. Pp. 276, D. Van Nostrand Company, Inc., Princeton, New Jersey, 1957. Price, $6.75.

It seems the purpose of a dictionary is to define or describe terms commonly and less commonly used in precise language and in a scholarly way. Since the use of a language is, to some extent, a unifying principle of human thought and activity, it appears highly questionable whether any scientific speciality should have its own dictionary. The authors of this dictionary attempted a compromise which seems not too successful in many respects. Definitions have been left incomplete by restricting them to usage in microbiology alone and omitting different meanings as used in other disciplines. For instance, "biochemistry" is limited to chemical processes of bacteria, and "organelles" are described as specialized structures of protozoa, while the usage of the word in general cytology has been omitted. These are minor shortcomings, but there are others of more serious nature, some of which are quoted below.

First, the language used is not precise and is frequently ambiguous. Quotations sometimes are wrong as in "Athlete's Foot" which is a dermatomycosis and not a dermatomycoses, or in "Strepto" which is derived from streptos and not from streptus. "Contagion" is defined as the communication of disease rather than as an agent of a communicable disease.

Second, quite frequently something else is given instead of definitions, e.g., "Stickland reaction." Here one finds the description of the fact that certain organisms obtain energy from amino acids whereas the description of the reaction itself would be appropriate. In other cases the definition is replaced by a recipe or an outline of a procedure.

Third, a number of definitions are definitely wrong. To quote only a few, the following examples are given: "Abscess" is not a pus cavity but a focus of acute inflammatory reaction associated with necrosis leading to pus formation; "anamnestic reaction" leads not only to a more rapid but also to a greater rise of the antibody titer; "chemotaxis" usually refers to the direct mobility of phagocytes toward microorganisms or other particulate matter rather than the motility of microorganisms themselves; "exotoxin" is characterized as being a better antigen than an endotoxin. This might be true but is misleading when it is given as the only differentiation between the two toxins. As a matter of fact, endotoxin is a good antigen, but the antibody formed does not neutralize its biologic activity as does an antitoxin; "hypertrophy" is usually not associated with an increase of the number of cells but represents an increase of the size of cells. Hyperplasia is the term used when the number of cells is predominantly increased; "Koch phenomen" does not refer to the reaction as a consequence of the injection of tuberculin but refers to the reaction to tubercle bacilli themselves when they are introduced in an already infected host; "*Mycobacterium avium*" is not a type of tuberculosis common in birds but is the agent causing avian tuberculosis, and "tuberculin" is not a substance from the filtrate of tubercle bacilli but rather a mixture of substances derived from a culture filtrate; "tumefaciens" means tumor producing and "V antigen" can in itself not be virulent but can only render strains of Salmonella virulent when present; and so on.

From the above quotations it becomes clear that this dictionary cannot be recommended either to the advanced worker or to the beginner. Popularization should not lead to incorrectness but to the application of definitions and terms understandable to the

non-specialist. The reviewer would not want any of his technicians to use this dictionary for reference because more accurate information can be obtained from existing dictionaries and from a text.

EMANUEL SUTER
Gainesville, Florida

BCG AND VOLE VACCINATION. By K. Neville Irvine. Second edition. Pp. 103 with illustrations, National Association for the Prevention of Tuberculosis, London, 1957. Price, $3.00.

This book was written mainly as a manual for doctors, nurses, and health visitors concerned with BCG vaccination and, in a very small number of words, gives an absolutely clear and practical guide to the problems and techniques involved. The general layout of the book is retained in the second edition, but there are very many minor alterations. What makes this book remarkable is that the author, while writing an account full of practical detail, has managed also to be thought-provoking and undogmatic. He has achieved this feat by including short theoretic discussions, by giving a useful list of references in each chapter, and by indicating some of the more recent work on the methods and results of BCG and vole vaccination.

The selection of controversial topics discussed is perhaps open to criticism. Thus, an account of De Assis' work on oral BCG is given, but the relationships between chemoprophylaxis and vaccination and Canetti's work on isoniazid-resistant BCG are not mentioned. Perhaps Dr. Irvine will include these topics, together with a comment on Lorber's recent work on BCG tuberculin, in the next edition of this excellent little book.

H. LAMBERT
New York, New York

BOOKS RECEIVED

ANTIBIOTICS ANNUAL 1957–1958. Edited by Henry Welch and Felix Marti-Ibanez. Pp. 1070, Medical Encyclopedia, Inc., New York, 1958. Price, $12.00.

THE BACTERIOLOGY OF TUBERCULOSIS. By Egons Darzins. Pp. 488, University of Minnesota Press, Minneapolis, 1958. Price, $10.00.

MODERN DRUG TREATMENT IN TUBERCULOSIS. By J. D. Ross. Pp. 47, National Association for the Prevention of Tuberculosis, London, 1958. Price 5s.

RESPIRATORY DISEASE AND THE GENERAL PRACTITIONER. By C. H. C. Toussaint. Pp. 28, National Association for the Prevention of Tuberculosis, London, 1958. Price, 7s.6d.

STUDIES ON PHAGOCYTIC STIMULATION. By B. Gözsy and L. Kátó. Pp. 135, Therien Frères Limitée, Montreal, 1957.

TRACHEOTOMY: A CLINICAL AND EXPERIMENTAL STUDY. By Thomas G. Nelson. Pp. 111, The Williams & Wilkins Company, Baltimore, 1958. Price, $3.75.

American Trudeau Society

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NOTICES

The following dates and locations of the next three annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 18–23
1959—Chicago, Illinois—May 24–29
1960—Los Angeles, California—May 15–20

ANNUAL MEETING OF GERONTOLOGICAL SOCIETY, INC. 1958

The eleventh annual scientific meeting of the Gerontological Society, Inc. will be held at the Bellevue-Stratford Hotel, Philadelphia, Pennsylvania, November 6–8, 1958.

Abstracts of papers for the program should be submitted to the Program Committee for consideration by July 1, 1958. Abstracts should be sent to the sub-chairmen of the section in which the author elects to give his paper.

The sub-chairmen are: *Clinical Medicine*—Dr. Ewald Busse, Duke University Hospital, Durham, North Carolina; *Biology*—Dr. Morris Rockstein, Department of Physiology, New York University, 550 First Avenue, New York 16, New York; *Psychology*—Dr. Ethel Shanas, National Opinion Research Center, 5711 South Woodlawn Avenue, Chicago, Illinois; and *Sociology*—Dr. W. M. Beattie, Jr., Department of Sociology, Washington University, St. Louis, Missouri.

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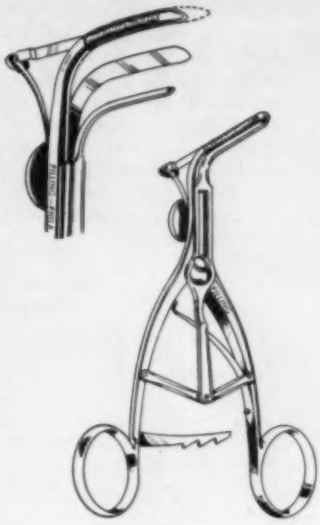

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Reference: Salvatore, J. E., Maloney, W. H.: "A New Rapid Tracheotomy Instrument," Journal American Medical Association, vol. 165, pp. 2077–78 (December 21, 1957)

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The ATS committee meetings will be held Friday, Saturday, and Sunday, May 16, 17, and 18.

Registration will begin Saturday afternoon, May 17, and continue through Sunday evening at the Bellevue-Stratford Hotel. Beginning Monday, May 19, registration, the exhibits program, and regular daytime sessions will be held at Convention Hall. Evening sessions through the week will be held at the Bellevue-Stratford.

Medical, public health, and nursing sessions will begin on Monday, May 19. General sessions will include an opening keynote program Monday A.M., the awards session Wednesday evening, and a session Thursday morning, May 22.

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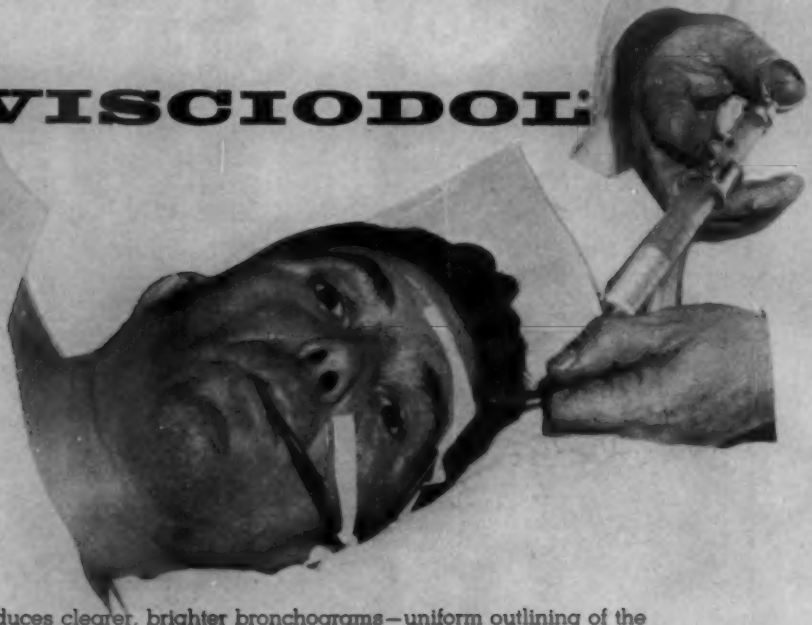

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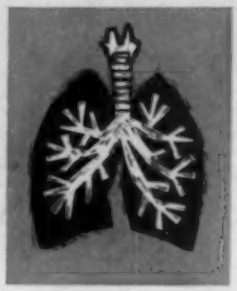

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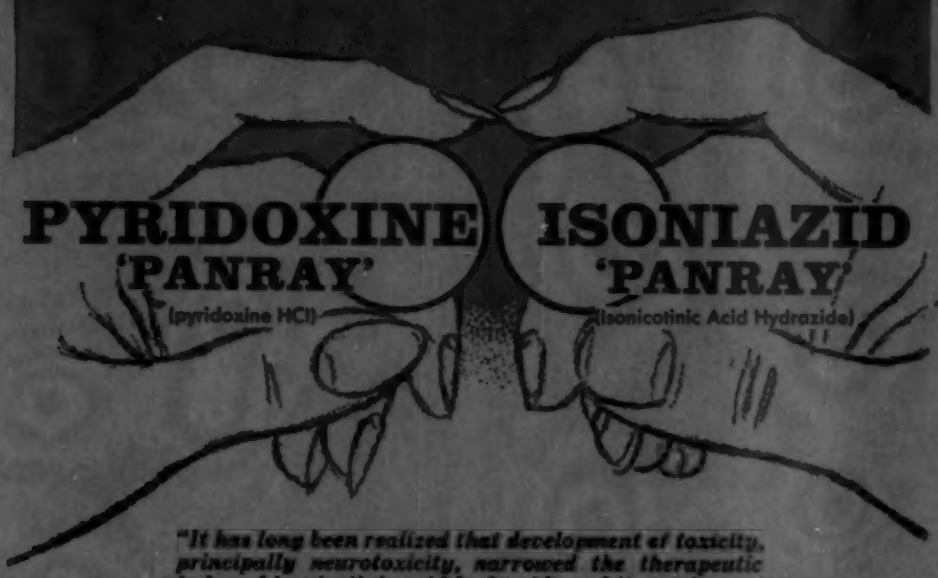

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